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<p>(54) Title: 20 HUMAN SECRETED PROTEINS  (57) Abstract  The present invention relates to 20 human secreted proteins and isolated nucleic acids containing the coding regions of the genes encoding such proteins. Also provided are vectors, host cells, antibodies, and recombinant methods for producing human secreted proteins. The invention further relates to diagnostic and therapeutic methods useful for diagnosing and treating disorders related to these human secreted proteins.</p>		

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## 20 Human Secreted Proteins

### *Field of the Invention*

This invention relates to newly identified polynucleotides and the polypeptides encoded by these polynucleotides, uses of such polynucleotides and polypeptides, and  
5 their production.

### *Background of the Invention*

Unlike bacterium, which exist as a single compartment surrounded by a membrane, human cells and other eucaryotes are subdivided by membranes into many functionally distinct compartments. Each membrane-bounded compartment, or  
10 organelle, contains different proteins essential for the function of the organelle. The cell uses "sorting signals," which are amino acid motifs located within the protein, to target proteins to particular cellular organelles.

One type of sorting signal, called a signal sequence, a signal peptide, or a leader sequence, directs a class of proteins to an organelle called the endoplasmic reticulum  
15 (ER). The ER separates the membrane-bounded proteins from all other types of proteins. Once localized to the ER, both groups of proteins can be further directed to another organelle called the Golgi apparatus. Here, the Golgi distributes the proteins to vesicles, including secretory vesicles, the cell membrane, lysosomes, and the other organelles.

20 Proteins targeted to the ER by a signal sequence can be released into the extracellular space as a secreted protein. For example, vesicles containing secreted proteins can fuse with the cell membrane and release their contents into the extracellular space - a process called exocytosis. Exocytosis can occur constitutively or after receipt of a triggering signal. In the latter case, the proteins are stored in secretory vesicles (or  
25 secretory granules) until exocytosis is triggered. Similarly, proteins residing on the cell membrane can also be secreted into the extracellular space by proteolytic cleavage of a "linker" holding the protein to the membrane.

Despite the great progress made in recent years, only a small number of genes encoding human secreted proteins have been identified. These secreted proteins include  
30 the commercially valuable human insulin, interferon, Factor VIII, human growth hormone, tissue plasminogen activator, and erythropoietin. Thus, in light of the pervasive role of secreted proteins in human physiology, a need exists for identifying and characterizing novel human secreted proteins and the genes that encode them. This knowledge will allow one to detect, to treat, and to prevent medical disorders by using  
35 secreted proteins or the genes that encode them.

### *Summary of the Invention*

The present invention relates to novel polynucleotides and the encoded polypeptides. Moreover, the present invention relates to vectors, host cells, antibodies, and recombinant methods for producing the polypeptides and polynucleotides. Also provided are diagnostic methods for detecting disorders related to the polypeptides, and therapeutic methods for treating such disorders. The invention further relates to screening methods for identifying binding partners of the polypeptides.

### *Detailed Description*

#### **Definitions**

The following definitions are provided to facilitate understanding of certain terms used throughout this specification.

In the present invention, "isolated" refers to material removed from its original environment (e.g., the natural environment if it is naturally occurring), and thus is altered "by the hand of man" from its natural state. For example, an isolated polynucleotide could be part of a vector or a composition of matter, or could be contained within a cell, and still be "isolated" because that vector, composition of matter, or particular cell is not the original environment of the polynucleotide.

In the present invention, a "secreted" protein refers to those proteins capable of being directed to the ER, secretory vesicles, or the extracellular space as a result of a signal sequence, as well as those proteins released into the extracellular space without necessarily containing a signal sequence. If the secreted protein is released into the extracellular space, the secreted protein can undergo extracellular processing to produce a "mature" protein. Release into the extracellular space can occur by many mechanisms, including exocytosis and proteolytic cleavage.

As used herein, a "polynucleotide" refers to a molecule having a nucleic acid sequence contained in SEQ ID NO:X or the cDNA contained within the clone deposited with the ATCC. For example, the polynucleotide can contain the nucleotide sequence of the full length cDNA sequence, including the 5' and 3' untranslated sequences, the coding region, with or without the signal sequence, the secreted protein coding region, as well as fragments, epitopes, domains, and variants of the nucleic acid sequence. Moreover, as used herein, a "polypeptide" refers to a molecule having the translated amino acid sequence generated from the polynucleotide as broadly defined.

In the present invention, the full length sequence identified as SEQ ID NO:X was often generated by overlapping sequences contained in multiple clones (contig



analysis). A representative clone containing all or most of the sequence for SEQ ID NO:X was deposited with the American Type Culture Collection ("ATCC"). As shown in Table 1, each clone is identified by a cDNA Clone ID (Identifier) and the ATCC Deposit Number. The ATCC is located at 10801 University Boulevard,  
5 Manassas, Virginia 20110-2209, USA. The ATCC deposit was made pursuant to the terms of the Budapest Treaty on the international recognition of the deposit of microorganisms for purposes of patent procedure.

A "polynucleotide" of the present invention also includes those polynucleotides capable of hybridizing, under stringent hybridization conditions, to sequences contained  
10 in SEQ ID NO:X, the complement thereof, or the cDNA within the clone deposited with the ATCC. "Stringent hybridization conditions" refers to an overnight incubation at 42° C in a solution comprising 50% formamide, 5x SSC (750 mM NaCl, 75 mM sodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 µg/ml denatured, sheared salmon sperm DNA, followed by washing the  
15 filters in 0.1x SSC at about 65°C.

Also contemplated are nucleic acid molecules that hybridize to the polynucleotides of the present invention at lower stringency hybridization conditions. Changes in the stringency of hybridization and signal detection are primarily accomplished through the manipulation of formamide concentration (lower percentages  
20 of formamide result in lowered stringency); salt conditions, or temperature. For example, lower stringency conditions include an overnight incubation at 37°C in a solution comprising 6X SSPE (20X SSPE = 3M NaCl; 0.2M NaH<sub>2</sub>PO<sub>4</sub>; 0.02M EDTA, pH 7.4), 0.5% SDS, 30% formamide, 100 ug/ml salmon sperm blocking DNA; followed by washes at 50°C with 1XSSPE, 0.1% SDS. In addition, to achieve even  
25 lower stringency, washes performed following stringent hybridization can be done at higher salt concentrations (e.g. 5X SSC).

Note that variations in the above conditions may be accomplished through the inclusion and/or substitution of alternate blocking reagents used to suppress background in hybridization experiments. Typical blocking reagents include  
30 Denhardt's reagent, BLOTTO, heparin, denatured salmon sperm DNA, and commercially available proprietary formulations. The inclusion of specific blocking reagents may require modification of the hybridization conditions described above, due to problems with compatibility.

Of course, a polynucleotide which hybridizes only to polyA+ sequences (such  
35 as any 3' terminal polyA+ tract of a cDNA shown in the sequence listing), or to a

complementary stretch of T (or U) residues, would not be included in the definition of "polynucleotide," since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone).

5           The polynucleotide of the present invention can be composed of any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. For example, polynucleotides can be composed of single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and  
10   double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, the polynucleotide can be composed of triple-stranded regions comprising RNA or DNA or both RNA and DNA. A polynucleotide may also contain one or more modified bases or DNA or RNA backbones modified for stability  
15   or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications can be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically, or metabolically modified forms.

          The polypeptide of the present invention can be composed of amino acids joined  
20   to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres, and may contain amino acids other than the 20 gene-encoded amino acids. The polypeptides may be modified by either natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs,  
25   as well as in a voluminous research literature. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be  
30   branched, for example, as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a  
35   nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine,

formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. (See, for instance, PROTEINS -  
5 STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993); POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, pgs. 1-12 (1983); Seifter et al., Meth Enzymol 182:626-646 (1990);  
10 Rattan et al., Ann NY Acad Sci 663:48-62 (1992).)

"SEQ ID NO:X" refers to a polynucleotide sequence while "SEQ ID NO:Y" refers to a polypeptide sequence, both sequences identified by an integer specified in Table 1.

"A polypeptide having biological activity" refers to polypeptides exhibiting  
15 activity similar, but not necessarily identical to, an activity of a polypeptide of the present invention, including mature forms, as measured in a particular biological assay, with or without dose dependency. In the case where dose dependency does exist, it need not be identical to that of the polypeptide, but rather substantially similar to the dose-dependence in a given activity as compared to the polypeptide of the present  
20 invention (i.e., the candidate polypeptide will exhibit greater activity or not more than about 25-fold less and, preferably, not more than about tenfold less activity, and most preferably, not more than about three-fold less activity relative to the polypeptide of the present invention.)

## 25 Polynucleotides and Polypeptides of the Invention

### FEATURES OF PROTEIN ENCODED BY GENE NO: 1

This gene is expressed primarily in brain and CD34 positive cells.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a  
30 biological sample and for diagnosis of diseases and conditions which include, but are not limited to, central nervous system (CNS) and immune-system diseases. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the CNS and immune  
35 system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., brain and other tissue of the nervous

system, blood cells, and cells and tissue of the immune system, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred epitopes include those comprising a sequence shown in SEQ ID NO:31 as residues: Asp-44 to Gly-49, Val-84 to Lys-90.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for diagnosing and treating CNS and immune-system diseases.

## FEATURES OF PROTEIN ENCODED BY GENE NO: 2

The translation product of this gene shares sequence homology with LIM domain proteins which are thought to be important in regulating cellular functions such as cell proliferation and differentiation. In particular, it is believed that this gene encodes the human ortholog of mouse testin. See, for example, Gene 156(2):283-286 (1995), which is incorporated herein by reference. LIM proteins are described in Proc. Natl. Acad. Sci. U.S.A. 90:4404-4408(1993), which is incorporated herein by reference. Based on the sequence similarity to other members of the LIM family, polypeptides encoded by this gene are expected to share certain biological activities with other LIM polypeptides, in particular mouse Testin. Preferred polypeptides encoded by this gene comprise the following amino acid sequence (LIM domain):

CAGCDELIFSNEYTQAENQNWHLKHFFCFDCDSIL (SEQ ID NO:51). Especially preferred polypeptides encoded by this gene comprise the following amino acid sequence: ARGFVCSTCHELLVDMIFYWKNEKLYCGRHYCD  
SEKPRCAGCDELIFSNEYTQAENQNWHLKHFFCFDCDSILAGEIYVMVNDKPV  
CKPCYVKNHAVVCQGCHNAIDPEVQRVTYNNFSWHASTECFLCSCCSKCLIG  
QKFMPVEGMVFCSVECKKRMS (SEQ ID NO:52). Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in testis and to a lesser extent in Hodgkin's lymphoma, T cell and adrenal gland tumor.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, immune diseases, reproductive disorders and cancers. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing

immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the reproductive and immune systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., testis and other reproductive tissue, lymphoid tissue, tissue and cells of the immune system, blood cells, and adrenal gland, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution and homology to LIM proteins indicates that polynucleotides and polypeptides corresponding to this gene are useful for treating diseases of the immune system and male reproductive system.

#### 15 **FEATURES OF PROTEIN ENCODED BY GENE NO: 3**

This gene is expressed primarily in infant brain, prostate, embryo and to a lesser extent in parathyroid, adrenal gland tumor, thymus.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, immune system and central nervous system (CNS) diseases. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system and CNS, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., brain and other tissue of the nervous system, tissue and cells of the immune system, differentiating tissue, parathyroid, adrenal gland, and thymus, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for treating and diagnosis of immune system and CNS diseases.

**FEATURES OF PROTEIN ENCODED BY GENE NO: 4**

This gene maps to chromosome 4 (Chr.4, D4S395-D4S414) and therefore polynucleotides of the present invention can be used in linkage analysis as a marker for chromosome 4.

5 This gene is expressed primarily in infant brain, embryo, parathyroid tumor and melanocyte and to a lesser extent in testis, chondrosarcoma, epididyma, placenta, endothelial cells and many other cell types, tissues and organs.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a  
10 biological sample and for diagnosis of diseases and conditions which include, but are not limited to, disorders of the nervous system, developmental related defects and abnormalities. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of  
15 the nervous system and immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., brain and other tissue of the nervous system, embryonic or differentiating cells or tissue, parathyroid, melanocytes, chondrocytes, testis and other reproductive tissue, placenta, and endothelial cells, and cancerous and wounded tissues) or bodily fluids (e.g.,  
20 serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred epitopes include those comprising a sequence shown in SEQ ID NO:34 as residues: Glu-28 to Thr-35.

25 The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for diagnosis and treatment of disorders of the nervous system, such as congenital malformations, degenerative diseases, trauma, inflammatory diseases, neoplasia, metabolic disorders, and immune diseases, particularly with T-cell involvement. The abundant expression in the parathyroid tumor  
30 indicates that protein products of this gene are useful in modulating calcium metabolism.

**FEATURES OF PROTEIN ENCODED BY GENE NO: 5**

35 The translation product of this gene shares sequence homology with ancient ubiquitous 46 kDa protein AUP46 precursor [*Mus musculus*] which is thought to be important in tissue and organ development.

This gene is expressed primarily in testes and placenta and to a lesser extent in fetal liver, brain, and activated T-cells.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, liver diseases and immunological disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and digestive system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., testes and other reproductive tissue, placenta, liver, brain and other tissue of the nervous system, and T-cells and other blood cells, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred epitopes include those comprising a sequence shown in SEQ ID NO:35 as residues: Pro-4 to Pro-9, Asp-14 to Gly-20, Arg-78 to His-87, Glu-161 to Gly-170, Leu-252 to Arg-258, Lys-269 to Pro-293, Asp-344 to Thr-349, Ser-379 to Gln-391, Arg-399 to Asp-410.

The tissue distribution and homology to AUP46 indicates that polynucleotides and polypeptides corresponding to this gene are useful for diagnosis and treatment of diseases in testes, placenta, liver, brain and activated T-cells, particularly diseases related to development of the organs associated with the foregoing tissues.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 6**

The translation product of this gene shares sequence homology with ATP7 region hypothetical protein which is thought to be important in development. This gene maps to chromosome 17 (D17S849-D17S796) and therefore polynucleotides of the present invention can be used in linkage analysis as a marker for chromosome 17.

This gene is expressed primarily in breast, brain and liver and to a lesser extent in prostate and thymus.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, breast cancer, brain tumor and liver cancer. Similarly, polypeptides and

antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and nerve system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues (e.g., developing tissue, mammary tissue, brain and other tissue of the nervous system, liver, prostate, and thymus, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 7

This gene is expressed primarily in liver, spleen, bone marrow and to a lesser extent in amygdala.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, liver, spleen diseases. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and digestive systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues (e.g., liver, spleen, bone marrow, cells and tissue of the immune system, and amygdala and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

#### 30 FEATURES OF PROTEIN ENCODED BY GENE NO: 8

Preferred mature polypeptides encoded by this gene comprise the following amino acid sequence:

GVARGHRDRGQASRRWLQEGGQCECKDWFLRAPRRKFMTVSGL  
PKKQCPCDHFKGNVKKTRHQRHHRKPNKHSRACQQFLKQCQLRSFALPL  
(SEQ ID NO:53).



This gene is expressed primarily in lung and to a lesser extent in pancreatic carcinoma and gall bladder.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, lung diseases and pancreatic carcinoma. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune, pulmonary and digestive systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues (e.g., lung, pancreas, and gall bladder, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred epitopes include those comprising a sequence shown in SEQ ID NO:38 as residues: Gly-31 to Gln-37.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 9**

This gene is expressed primarily in rhabdomyosarcoma and pituitary, and, to a lesser extent, in fetal lung and keratinocytes.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, rhabdomyosarcoma and disorders of the endocrine system or other endocrinopathies, including, but not limited to, endocrine polyglandular syndrome, endocrinoma, endocrine ophthalmopathy, and any of the great number of disease states and disorders which are caused by or relate to the abnormal secretion of factors originating in the endocrine gland. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and musculoskeletal system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., skeletal muscle, pituitary, endocrine glands, lung and keratinocytes, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e.,

the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred epitopes include those comprising a sequence shown in SEQ ID NO:39 as residues: Pro-34 to Phe-40.

#### 5 FEATURES OF PROTEIN ENCODED BY GENE NO: 10

This gene is expressed primarily in endometrial tumor, osteoblasts, and smooth muscle, and, to a lesser extent, in osteoclastoma, heart, and lung.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, endometrial tumor, osteoclastoma, and other bone remodeling disorders, and heart and lung diseases. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and bone systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues (e.g., endometrium, bone, heart and other cardiovascular tissue, lung and other pulmonary tissue, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred epitopes include those comprising a sequence shown in SEQ ID NO:40 as residues: Thr-33 to Arg-40.

#### 25 FEATURES OF PROTEIN ENCODED BY GENE NO: 11

This gene is expressed primarily in meningioma and dermatofibrosarcoma.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, fibrotic and neoplastic conditions of skin, connective tissue and other mesenchymal organs. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues (e.g., meninx, liver, skin, and vascular tissue, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal

fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred epitopes include those comprising a sequence shown in SEQ ID NO:41 as residues: Gln-50 to Met-56.

5 Tissue distribution of this gene indicates that it may be useful in the study, treatment, and diagnosis of fibrotic disorders and neoplasms of skin, liver, and other tissues.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 12**

10 This gene is expressed primarily in placenta, and colon cancer, and to a lesser extent in adult lung and brain frontal cortex.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, cancer. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system and neural system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues (e.g., placenta, colon, cells and tissue of the immune system, lung and other pulmonary tissue, brain and other tissue of the nervous system, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred epitopes include those comprising a sequence shown in SEQ ID NO:42 as residues: Met-1 to His-9, Arg-31 to Gly-38, Gly-102 to Trp-108.

25 The tissue distribution indicates that the protein product of this gene is useful for the treatment of neoplasia.

30

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 13**

This gene is expressed primarily in thymus, bone marrow, T-cells, macrophages, and, to a lesser extent, in breast and testes.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are

35

not limited to, cancer, autoimmune diseases, bone diseases. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., thymus, bone, T-cells and other blood cells, mammary tissue, and testes and other reproductive tissue, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred epitopes include those comprising a sequence shown in SEQ ID NO:43 as residues: Pro-36 to Trp-42, Arg-48 to Trp-56, Ser-58 to Ser-67.

#### 15 **FEATURES OF PROTEIN ENCODED BY GENE NO: 14**

This gene is expressed primarily in breast cancer, pituitary, and activated T-cells, and, to a lesser extent, in frontal cortex and breast.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, breast cancer, growth, and immune disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and neural system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., mammary tissue, pituitary, T-cells and other blood cells, cells and tissue of the immune system, brain and other tissue of the nervous system, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution indicates that the protein product of this gene is useful for diagnosis or treatment of breast cancer and growth disorders.

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**FEATURES OF PROTEIN ENCODED BY GENE NO: 15**

This gene is primarily expressed exclusively in neutrophils.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, lymphoma and bacterial infection. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, and more particularly, in neutrophils, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues and cell types (e.g., lymphoid tissue, hematopoietic tissue, neutrophils and other blood cells, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution indicates that the protein product of this gene is useful for treatment of lymphomas and various hematopoietic disorders, as well as in the diagnosis and treatment of bacterial infections, sepsis, and in disorders which particularly involve neutrophils.

**FEATURES OF PROTEIN ENCODED BY GENE NO: 16**

In specific embodiments, polypeptides of the invention comprise the sequence: HTQVEFIPRMQC (SEQ ID NO:54), LKIRKPINVITYHINRL (SEQ ID NO:55), RKMGIERNFHQSGKGI (SEQ ID NO:56), KVPTANIILNGERLNAFPIRT (SEQ ID NO:57), MYFLSSLLIHEHVISVIFSIL (SEQ ID NO:60), and/or IFSSVLHSFQYTNPV PFFFRFTPSTLFF (SEQ ID NO:58). Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in neutrophils only.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, lymphomas. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or

lower levels may be routinely detected in certain tissues (e.g., lymphoid tissue, hematopoietic tissue, neutrophils and other blood cells, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to  
5 the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution indicates that the protein product of this gene is useful for treatment of lymphomas and a variety of hematopoietic disorders.

#### 10 **FEATURES OF PROTEIN ENCODED BY GENE NO: 17**

This gene maps to chromosome 19 and therefore polynucleotides of the present invention can be used in linkage analysis as a marker for chromosome 19.

This gene is expressed primarily in prostate cancer, adult lung and adult pulmonary and to a lesser extent in prostate and adrenal gland tumor.

15 Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, prostate cancer and endocrine disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes  
20 for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the endocrine system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues (e.g., prostate, lung and other pulmonary tissue, endocrine tissue, adrenal gland, cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial  
25 fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred epitopes include those comprising a sequence shown in SEQ ID NO:47 as residues: Gly-2 to Gly-9, Thr-39 to Arg-47.

30 The tissue distribution indicates that the protein product of this gene is useful for diagnosis or treatment of prostate cancer, prostate disorders and endocrine disorders.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 18**

This sequence shares high degree homology with the UFO oncoprotein. This  
35 protein is a tyrosine kinase receptor. While the functions of this UFO receptor are unknown, it is known that the receptor plays a role in tumorigenesis.

This gene is expressed primarily in L8 cell line.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, cancer. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred epitopes include those comprising a sequence shown in SEQ ID NO:48 as residues: Ala-37 to Ser-49.

The tissue distribution and homology with the UFO oncoprotein indicates that the protein product of this gene is useful for the treatment of cancer.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 19**

This gene is expressed primarily in L8 cell line.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, cancer and the immune system. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system], expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution indicates that the protein product of this gene is useful for the treatment of cancer.

**FEATURES OF PROTEIN ENCODED BY GENE NO: 20**

In specific embodiments, polypeptides of the invention comprise the sequence EDGSAPREGETSAPRLPEVVRRITSAGIC (SEQ ID NO:61). Polynucleotides encoding these polypeptides are also encompassed by the invention.

5        This gene is expressed primarily in A1 and A14 cell lines.

         Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, cancer. Similarly, polypeptides and antibodies directed to these  
10       polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) or  
15       another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

         The tissue distribution indicates that the protein product of this gene is useful for the diagnosis and treatment of cancer.



Gene No.	cDNA Clone ID	ATCC Deposit Nr and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
1	HCWHZ93	97955 03/13/97 209074 05/22/97	ZAP Express	11	919	99	919	205	205	31	1	32	33	94
2	HDTAY29	97955 03/13/97 209074 05/22/97	pCMVSPORT 2.0	12	1026	57	588	363	363	32	1	22	23	23
3	HE2FV03	97955 03/13/97 209074 05/22/97	Uni-ZAP XR	13	2067	1	1251	116	116	33	1	21	22	42
4	HE8ND56	97955 03/13/97 209074 05/22/97	Uni-ZAP XR	14	1341	372	1304	530	530	34	1	25	26	71
5	HMQBU44	97955 03/13/97 209074 05/22/97	Uni-ZAP XR	15	1443	8	1431	27	27	35	1	37	38	410
6	HHGAS83	97955 03/13/97 209074 05/22/97	Lambda ZAP II	16	654	141	653	318	318	36	1	21	22	25

Gene No.	cDNA Clone ID	ATCC Deposit Nr and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
7	HIPDJ64	97955 03/13/97 209074 05/22/97	Uni-ZAP XR	17	749	378	749	583	583	37	1	18	19	37
8	HLHCM89	97955 03/13/97 209074 05/22/97	Uni-ZAP XR	18	511	141	511	233	233	38	1	26	27	64
9	HLHEF26	97955 03/13/97 209074 05/22/97	Uni-ZAP XR	19	689	1	689	92	92	39	1	19	20	155
10	HLHEO50	97955 03/13/97 209074 05/22/97	Uni-ZAP XR	20	1147	69	1119	171	171	40	1	18	19	119
11	HMJAG94	97955 03/13/97 209074 05/22/97	pSport1	21	532	1	532	64	64	41	1	24	25	56
12	HSKNK73	97955 03/13/97 209074 05/22/97	pBluescript	22	2743	646	1368	750	750	42	1	34	35	109

Gene No.	cDNA Clone ID	ATCC Deposit Nr and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
13	HSSMS41	97955 03/13/97 209074 05/22/97	Uni-ZAP XR	23	820	192	820	328	328	43	1	22	23	69
14	HNGBV36	97955 03/13/97 209074 05/22/97	Uni-ZAP XR	24	995	6	555	69	69	44	1	32	33	67
15	HNGDE27	97955 03/13/97 209074 05/22/97	Uni-ZAP XR	25	649	1	649	140	140	45	1	17	18	41
16	HNGDQ28	97955 03/13/97 209074 05/22/97	Uni-ZAP XR	26	979	1	979	148	148	46	1			9
17	HPFDU90	97955 03/13/97 209074 05/22/97	Uni-ZAP XR	27	905	454	905	541	541	47	1	27	28	47
18	HRLMD77	97955 03/13/97 209074 05/22/97	ZAP Express	28	299	1	299	153	153	48	1	23	24	49

Gene No.	cDNA Clone ID	ATCC Deposit Nr and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
19	HRLMF92	97955 03/13/97 209074 05/22/97	ZAP Express	29	338	1	338	154	154	49	1	24	25	46
20	HRSMF51	97955 03/13/97 209074 05/22/97	ZAP Express	30	500	1	500	225	225	50	1	29	30	30

Table 1 summarizes the information corresponding to each "Gene No." described above. The nucleotide sequence identified as "NT SEQ ID NO:X" was assembled from partially homologous ("overlapping") sequences obtained from the "cDNA clone ID" identified in Table 1 and, in some cases, from additional related DNA clones. The overlapping sequences were assembled into a single contiguous sequence of high redundancy (usually three to five overlapping sequences at each nucleotide position), resulting in a final sequence identified as SEQ ID NO:X.

The cDNA Clone ID was deposited on the date and given the corresponding deposit number listed in "ATCC Deposit No:Z and Date." Some of the deposits contain multiple different clones corresponding to the same gene. "Vector" refers to the type of vector contained in the cDNA Clone ID.

"Total NT Seq." refers to the total number of nucleotides in the contig identified by "Gene No." The deposited clone may contain all or most of these sequences, reflected by the nucleotide position indicated as "5' NT of Clone Seq." and the "3' NT of Clone Seq." of SEQ ID NO:X. The nucleotide position of SEQ ID NO:X of the putative start codon (methionine) is identified as "5' NT of Start Codon." Similarly, the nucleotide position of SEQ ID NO:X of the predicted signal sequence is identified as "5' NT of First AA of Signal Pep."

The translated amino acid sequence, beginning with the methionine, is identified as "AA SEQ ID NO:Y," although other reading frames can also be easily translated using known molecular biology techniques. The polypeptides produced by these alternative open reading frames are specifically contemplated by the present invention.

The first and last amino acid position of SEQ ID NO:Y of the predicted signal peptide is identified as "First AA of Sig Pep" and "Last AA of Sig Pep." The predicted first amino acid position of SEQ ID NO:Y of the secreted portion is identified as "Predicted First AA of Secreted Portion." Finally, the amino acid position of SEQ ID NO:Y of the last amino acid in the open reading frame is identified as "Last AA of ORF."

SEQ ID NO:X and the translated SEQ ID NO:Y are sufficiently accurate and otherwise suitable for a variety of uses well known in the art and described further below. For instance, SEQ ID NO:X is useful for designing nucleic acid hybridization probes that will detect nucleic acid sequences contained in SEQ ID NO:X or the cDNA contained in the deposited clone. These probes will also hybridize to nucleic acid molecules in biological samples, thereby enabling a variety of forensic and diagnostic methods of the invention. Similarly, polypeptides identified from SEQ ID NO:Y may be used to generate antibodies which bind specifically to the secreted proteins encoded by the cDNA clones identified in Table 1.

Nevertheless, DNA sequences generated by sequencing reactions can contain sequencing errors. The errors exist as misidentified nucleotides, or as insertions or deletions of nucleotides in the generated DNA sequence. The erroneously inserted or deleted nucleotides cause frame shifts in the reading frames of the predicted amino acid sequence. In these cases, the predicted amino acid sequence diverges from the actual amino acid sequence, even though the generated DNA sequence may be greater than 99.9% identical to the actual DNA sequence (for example, one base insertion or deletion in an open reading frame of over 1000 bases).

Accordingly, for those applications requiring precision in the nucleotide sequence or the amino acid sequence, the present invention provides not only the generated nucleotide sequence identified as SEQ ID NO:X and the predicted translated amino acid sequence identified as SEQ ID NO:Y, but also a sample of plasmid DNA containing a human cDNA of the invention deposited with the ATCC, as set forth in Table 1. The nucleotide sequence of each deposited clone can readily be determined by sequencing the deposited clone in accordance with known methods. The predicted amino acid sequence can then be verified from such deposits. Moreover, the amino acid sequence of the protein encoded by a particular clone can also be directly determined by peptide sequencing or by expressing the protein in a suitable host cell containing the deposited human cDNA, collecting the protein, and determining its sequence.

The present invention also relates to the genes corresponding to SEQ ID NO:X, SEQ ID NO:Y, or the deposited clone. The corresponding gene can be isolated in accordance with known methods using the sequence information disclosed herein. Such methods include preparing probes or primers from the disclosed sequence and identifying or amplifying the corresponding gene from appropriate sources of genomic material.

Also provided in the present invention are species homologs. Species homologs may be isolated and identified by making suitable probes or primers from the sequences provided herein and screening a suitable nucleic acid source for the desired homologue.

The polypeptides of the invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

The polypeptides may be in the form of the secreted protein, including the mature form, or may be a part of a larger protein, such as a fusion protein (see below).

It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification, such as multiple histidine residues, or an additional sequence for stability during recombinant production.

- 5        The polypeptides of the present invention are preferably provided in an isolated form, and preferably are substantially purified. A recombinantly produced version of a polypeptide, including the secreted polypeptide, can be substantially purified by the one-step method described in Smith and Johnson, Gene 67:31-40 (1988). Polypeptides of the invention also can be purified from natural or recombinant sources  
10        using antibodies of the invention raised against the secreted protein in methods which are well known in the art.

### **Signal Sequences**

- 15        Methods for predicting whether a protein has a signal sequence, as well as the cleavage point for that sequence, are available. For instance, the method of McGeoch, Virus Res. 3:271-286 (1985), uses the information from a short N-terminal charged region and a subsequent uncharged region of the complete (uncleaved) protein. The method of von Heinje, Nucleic Acids Res. 14:4683-4690 (1986) uses the information  
20        from the residues surrounding the cleavage site, typically residues -13 to +2, where +1 indicates the amino terminus of the secreted protein. The accuracy of predicting the cleavage points of known mammalian secretory proteins for each of these methods is in the range of 75-80%. (von Heinje, supra.) However, the two methods do not always produce the same predicted cleavage point(s) for a given protein.

- 25        In the present case, the deduced amino acid sequence of the secreted polypeptide was analyzed by a computer program called SignalP (Henrik Nielsen et al., Protein Engineering 10:1-6 (1997)), which predicts the cellular location of a protein based on the amino acid sequence. As part of this computational prediction of localization, the methods of McGeoch and von Heinje are incorporated. The analysis of the amino acid sequences of the secreted proteins described herein by this program provided the results  
30        shown in Table 1.

- As one of ordinary skill would appreciate, however, cleavage sites sometimes vary from organism to organism and cannot be predicted with absolute certainty. Accordingly, the present invention provides secreted polypeptides having a sequence shown in SEQ ID NO:Y which have an N-terminus beginning within 5 residues (i.e., +  
35        or - 5 residues) of the predicted cleavage point. Similarly, it is also recognized that in some cases, cleavage of the signal sequence from a secreted protein is not entirely

uniform, resulting in more than one secreted species. These polypeptides, and the polynucleotides encoding such polypeptides, are contemplated by the present invention.

Moreover, the signal sequence identified by the above analysis may not necessarily predict the naturally occurring signal sequence. For example, the naturally occurring signal sequence may be further upstream from the predicted signal sequence. However, it is likely that the predicted signal sequence will be capable of directing the secreted protein to the ER. These polypeptides, and the polynucleotides encoding such polypeptides, are contemplated by the present invention.

#### 10 **Polynucleotide and Polypeptide Variants**

"Variant" refers to a polynucleotide or polypeptide differing from the polynucleotide or polypeptide of the present invention, but retaining essential properties thereof. Generally, variants are overall closely similar, and, in many regions, identical to the polynucleotide or polypeptide of the present invention.

15 By a polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence of the present invention, it is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence encoding the polypeptide. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. The query sequence may be an entire sequence shown in Table 1, the ORF (open reading frame), or any fragment specified as described herein.

25 As a practical matter, whether any particular nucleic acid molecule or polypeptide is at least 90%, 95%, 96%, 97%, 98% or 99% identical to a nucleotide sequence of the present invention can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci. (1990) 6:237-245). In a sequence alignment the query and subject sequences are both DNA sequences. An RNA sequence can be compared by converting U's to T's. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB alignment of DNA sequences to calculate percent identity are:

35 Matrix=Unitary, k-tuple=4, Mismatch Penalty=1, Joining Penalty=30, Randomization



Group Length=0, Cutoff Score=1, Gap Penalty=5, Gap Size Penalty 0.05, Window Size=500 or the length of the subject nucleotide sequence, whichever is shorter.

If the subject sequence is shorter than the query sequence because of 5' or 3' deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for 5' and 3' truncations of the subject sequence when calculating percent identity. For subject sequences truncated at the 5' or 3' ends, relative to the query sequence, the percent identity is corrected by calculating the number of bases of the query sequence that are 5' and 3' of the subject sequence, which are not matched/aligned, as a percent of the total bases of the query sequence. Whether a nucleotide is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This corrected score is what is used for the purposes of the present invention. Only bases outside the 5' and 3' bases of the subject sequence, as displayed by the FASTDB alignment, which are not matched/aligned with the query sequence, are calculated for the purposes of manually adjusting the percent identity score.

For example, a 90 base subject sequence is aligned to a 100 base query sequence to determine percent identity. The deletions occur at the 5' end of the subject sequence and therefore, the FASTDB alignment does not show a matched/alignment of the first 10 bases at 5' end. The 10 unpaired bases represent 10% of the sequence (number of bases at the 5' and 3' ends not matched/total number of bases in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 bases were perfectly matched the final percent identity would be 90%. In another example, a 90 base subject sequence is compared with a 100 base query sequence. This time the deletions are internal deletions so that there are no bases on the 5' or 3' of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only bases 5' and 3' of the subject sequence which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to be made for the purposes of the present invention.

By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a query amino acid sequence of the present invention, it is intended that the amino acid sequence of the subject polypeptide is identical to the query sequence except that the subject polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the query amino acid sequence. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a query

amino acid sequence, up to 5% of the amino acid residues in the subject sequence may be inserted, deleted, (indels) or substituted with another amino acid. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular polypeptide is at least 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, the amino acid sequences shown in Table 1 or to the amino acid sequence encoded by deposited DNA clone can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci. (1990) 6:237-245). In a sequence alignment the query and subject sequences are either both nucleotide sequences or both amino acid sequences. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB amino acid alignment are: Matrix=PAM 0, k-tuple=2, Mismatch Penalty=1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=1, Window Size=sequence length, Gap Penalty=5, Gap Size Penalty=0.05, Window Size=500 or the length of the subject amino acid sequence, whichever is shorter.

If the subject sequence is shorter than the query sequence due to N- or C-terminal deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for N- and C-terminal truncations of the subject sequence when calculating global percent identity. For subject sequences truncated at the N- and C-termini, relative to the query sequence, the percent identity is corrected by calculating the number of residues of the query sequence that are N- and C-terminal of the subject sequence, which are not matched/aligned with a corresponding subject residue, as a percent of the total bases of the query sequence. Whether a residue is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This final percent identity score is what is used for the purposes of the present invention. Only residues to the N- and C-termini of the subject sequence, which are not matched/aligned with the query sequence, are considered for the purposes of manually adjusting the percent identity score. That is, only query residue positions outside the farthest N- and C-terminal residues of the subject sequence.

For example, a 90 amino acid residue subject sequence is aligned with a 100 residue query sequence to determine percent identity. The deletion occurs at the N-terminus of the subject sequence and therefore, the FASTDB alignment does not show a matching/alignment of the first 10 residues at the N-terminus. The 10 unpaired  
5 residues represent 10% of the sequence (number of residues at the N- and C- termini not matched/total number of residues in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 residues were perfectly matched the final percent identity would be 90%. In another example, a 90 residue subject sequence is compared with a 100 residue query sequence.  
10 This time the deletions are internal deletions so there are no residues at the N- or C-termini of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only residue positions outside the N- and C-terminal ends of the subject sequence, as displayed in the FASTDB alignment, which are not matched/aligned with the query  
15 sequence are manually corrected for. No other manual corrections are to made for the purposes of the present invention.

The variants may contain alterations in the coding regions, non-coding regions, or both. Especially preferred are polynucleotide variants containing alterations which produce silent substitutions, additions, or deletions, but do not alter the properties or  
20 activities of the encoded polypeptide. Nucleotide variants produced by silent substitutions due to the degeneracy of the genetic code are preferred. Moreover, variants in which 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination are also preferred. Polynucleotide variants can be produced for a variety of reasons, e.g., to optimize codon expression for a particular host (change codons in  
25 the human mRNA to those preferred by a bacterial host such as *E. coli*).

Naturally occurring variants are called "allelic variants," and refer to one of several alternate forms of a gene occupying a given locus on a chromosome of an organism. (Genes II, Lewin, B., ed., John Wiley & Sons, New York (1985).) These allelic variants can vary at either the polynucleotide and/or polypeptide level.  
30 Alternatively, non-naturally occurring variants may be produced by mutagenesis techniques or by direct synthesis.

Using known methods of protein engineering and recombinant DNA technology, variants may be generated to improve or alter the characteristics of the polypeptides of the present invention. For instance, one or more amino acids can be  
35 deleted from the N-terminus or C-terminus of the secreted protein without substantial loss of biological function. The authors of Ron et al., J. Biol. Chem. 268: 2984-2988 (1993), reported variant KGF proteins having heparin binding activity even after

deleting 3, 8, or 27 amino-terminal amino acid residues. Similarly, Interferon gamma exhibited up to ten times higher activity after deleting 8-10 amino acid residues from the carboxy terminus of this protein. (Dobeli et al., J. Biotechnology 7:199-216 (1988).)

Moreover, ample evidence demonstrates that variants often retain a biological activity similar to that of the naturally occurring protein. For example, Gayle and coworkers (J. Biol. Chem 268:22105-22111 (1993)) conducted extensive mutational analysis of human cytokine IL-1a. They used random mutagenesis to generate over 3,500 individual IL-1a mutants that averaged 2.5 amino acid changes per variant over the entire length of the molecule. Multiple mutations were examined at every possible amino acid position. The investigators found that "[m]ost of the molecule could be altered with little effect on either [binding or biological activity]." (See, Abstract.) In fact, only 23 unique amino acid sequences, out of more than 3,500 nucleotide sequences examined, produced a protein that significantly differed in activity from wild-type.

Furthermore, even if deleting one or more amino acids from the N-terminus or C-terminus of a polypeptide results in modification or loss of one or more biological functions, other biological activities may still be retained. For example, the ability of a deletion variant to induce and/or to bind antibodies which recognize the secreted form will likely be retained when less than the majority of the residues of the secreted form are removed from the N-terminus or C-terminus. Whether a particular polypeptide lacking N- or C-terminal residues of a protein retains such immunogenic activities can readily be determined by routine methods described herein and otherwise known in the art.

Thus, the invention further includes polypeptide variants which show substantial biological activity. Such variants include deletions, insertions, inversions, repeats, and substitutions selected according to general rules known in the art so as to have little effect on activity. For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie, J. U. et al., Science 247:1306-1310 (1990), wherein the authors indicate that there are two main strategies for studying the tolerance of an amino acid sequence to change.

The first strategy exploits the tolerance of amino acid substitutions by natural selection during the process of evolution. By comparing amino acid sequences in different species, conserved amino acids can be identified. These conserved amino acids are likely important for protein function. In contrast, the amino acid positions where substitutions have been tolerated by natural selection indicates that these positions are not critical for protein function. Thus, positions tolerating amino acid substitution could be modified while still maintaining biological activity of the protein.

The second strategy uses genetic engineering to introduce amino acid changes at specific positions of a cloned gene to identify regions critical for protein function. For example, site directed mutagenesis or alanine-scanning mutagenesis (introduction of single alanine mutations at every residue in the molecule) can be used. (Cunningham and Wells, Science 244:1081-1085 (1989).) The resulting mutant molecules can then be tested for biological activity.

As the authors state, these two strategies have revealed that proteins are surprisingly tolerant of amino acid substitutions. The authors further indicate which amino acid changes are likely to be permissive at certain amino acid positions in the protein. For example, most buried (within the tertiary structure of the protein) amino acid residues require nonpolar side chains, whereas few features of surface side chains are generally conserved. Moreover, tolerated conservative amino acid substitutions involve replacement of the aliphatic or hydrophobic amino acids Ala, Val, Leu and Ile; replacement of the hydroxyl residues Ser and Thr; replacement of the acidic residues Asp and Glu; replacement of the amide residues Asn and Gln, replacement of the basic residues Lys, Arg, and His; replacement of the aromatic residues Phe, Tyr, and Trp, and replacement of the small-sized amino acids Ala, Ser, Thr, Met, and Gly.

Besides conservative amino acid substitution, variants of the present invention include (i) substitutions with one or more of the non-conserved amino acid residues, where the substituted amino acid residues may or may not be one encoded by the genetic code, or (ii) substitution with one or more of amino acid residues having a substituent group, or (iii) fusion of the mature polypeptide with another compound, such as a compound to increase the stability and/or solubility of the polypeptide (for example, polyethylene glycol), or (iv) fusion of the polypeptide with additional amino acids, such as an IgG Fc fusion region peptide, or leader or secretory sequence, or a sequence facilitating purification. Such variant polypeptides are deemed to be within the scope of those skilled in the art from the teachings herein.

For example, polypeptide variants containing amino acid substitutions of charged amino acids with other charged or neutral amino acids may produce proteins with improved characteristics, such as less aggregation. Aggregation of pharmaceutical formulations both reduces activity and increases clearance due to the aggregate's immunogenic activity. (Pinckard et al., Clin. Exp. Immunol. 2:331-340 (1967); Robbins et al., Diabetes 36: 838-845 (1987); Cleland et al., Crit. Rev. Therapeutic Drug Carrier Systems 10:307-377 (1993).)

#### Polynucleotide and Polypeptide Fragments

In the present invention, a "polynucleotide fragment" refers to a short polynucleotide having a nucleic acid sequence contained in the deposited clone or shown in SEQ ID NO:X. The short nucleotide fragments are preferably at least about 15 nt, and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably, at least about 40 nt in length. A fragment "at least 20 nt in length," for example, is intended to include 20 or more contiguous bases from the cDNA sequence contained in the deposited clone or the nucleotide sequence shown in SEQ ID NO:X. These nucleotide fragments are useful as diagnostic probes and primers as discussed herein. Of course, larger fragments (e.g., 50, 150, 500, 600, 2000 nucleotides) are preferred.

Moreover, representative examples of polynucleotide fragments of the invention, include, for example, fragments having a sequence from about nucleotide number 1-50, 51-100, 101-150, 151-200, 201-250, 251-300, 301-350, 351-400, 401-450, 451-500, 501-550, 551-600, 651-700, or 701 to the end of SEQ ID NO:X or the cDNA contained in the deposited clone. In this context "about" includes the particularly recited ranges, larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. Preferably, these fragments encode a polypeptide which has biological activity.

In the present invention, a "polypeptide fragment" refers to a short amino acid sequence contained in SEQ ID NO:Y or encoded by the cDNA contained in the deposited clone. Protein fragments may be "free-standing," or comprised within a larger polypeptide of which the fragment forms a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments from about amino acid number 1-20, 21-40, 41-60, 61-80, 81-100, 102-120, 121-140, 141-160, or 161 to the end of the coding region. Moreover, polypeptide fragments can be about 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, or 150 amino acids in length. In this context "about" includes the particularly recited ranges, larger or smaller by several (5, 4, 3, 2, or 1) amino acids, at either extreme or at both extremes.

Preferred polypeptide fragments include the secreted protein as well as the mature form. Further preferred polypeptide fragments include the secreted protein or the mature form having a continuous series of deleted residues from the amino or the carboxy terminus, or both. For example, any number of amino acids, ranging from 1-60, can be deleted from the amino terminus of either the secreted polypeptide or the mature form. Similarly, any number of amino acids, ranging from 1-30, can be deleted from the carboxy terminus of the secreted protein or mature form. Furthermore, any combination of the above amino and carboxy terminus deletions are preferred.

Similarly, polynucleotide fragments encoding these polypeptide fragments are also preferred.

Also preferred are polypeptide and polynucleotide fragments characterized by structural or functional domains, such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions. Polypeptide fragments of SEQ ID NO:Y falling within conserved domains are specifically contemplated by the present invention. Moreover, polynucleotide fragments encoding these domains are also contemplated.

Other preferred fragments are biologically active fragments. Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of the polypeptide of the present invention. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity.

#### **Epitopes & Antibodies**

In the present invention, "epitopes" refer to polypeptide fragments having antigenic or immunogenic activity in an animal, especially in a human. A preferred embodiment of the present invention relates to a polypeptide fragment comprising an epitope, as well as the polynucleotide encoding this fragment. A region of a protein molecule to which an antibody can bind is defined as an "antigenic epitope." In contrast, an "immunogenic epitope" is defined as a part of a protein that elicits an antibody response. (See, for instance, Geysen et al., Proc. Natl. Acad. Sci. USA 81:3998-4002 (1983).)

Fragments which function as epitopes may be produced by any conventional means. (See, e.g., Houghten, R. A., Proc. Natl. Acad. Sci. USA 82:5131-5135 (1985) further described in U.S. Patent No. 4,631,211.)

In the present invention, antigenic epitopes preferably contain a sequence of at least seven, more preferably at least nine, and most preferably between about 15 to about 30 amino acids. Antigenic epitopes are useful to raise antibodies, including monoclonal antibodies, that specifically bind the epitope. (See, for instance, Wilson et al., Cell 37:767-778 (1984); Sutcliffe, J. G. et al., Science 219:660-666 (1983).)

Similarly, immunogenic epitopes can be used to induce antibodies according to methods well known in the art. (See, for instance, Sutcliffe et al., supra; Wilson et al., supra; Chow, M. et al., Proc. Natl. Acad. Sci. USA 82:910-914; and Bittle, F. J. et al., J. Gen. Virol. 66:2347-2354 (1985).) A preferred immunogenic epitope includes

the secreted protein. The immunogenic epitopes may be presented together with a carrier protein, such as an albumin, to an animal system (such as rabbit or mouse) or, if it is long enough (at least about 25 amino acids), without a carrier. However, immunogenic epitopes comprising as few as 8 to 10 amino acids have been shown to be  
5 sufficient to raise antibodies capable of binding to, at the very least, linear epitopes in a denatured polypeptide (e.g., in Western blotting.)

As used herein, the term "antibody" (Ab) or "monoclonal antibody" (Mab) is meant to include intact molecules as well as antibody fragments (such as, for example, Fab and F(ab')<sub>2</sub> fragments) which are capable of specifically binding to protein. Fab  
10 and F(ab')<sub>2</sub> fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding than an intact antibody. (Wahl et al., J. Nucl. Med. 24:316-325 (1983).) Thus, these fragments are preferred, as well as the products of a FAB or other immunoglobulin expression library. Moreover, antibodies of the present invention include chimeric, single chain, and  
15 humanized antibodies.

### **Fusion Proteins**

Any polypeptide of the present invention can be used to generate fusion proteins. For example, the polypeptide of the present invention, when fused to a  
20 second protein, can be used as an antigenic tag. Antibodies raised against the polypeptide of the present invention can be used to indirectly detect the second protein by binding to the polypeptide. Moreover, because secreted proteins target cellular locations based on trafficking signals, the polypeptides of the present invention can be used as targeting molecules once fused to other proteins.

25 Examples of domains that can be fused to polypeptides of the present invention include not only heterologous signal sequences, but also other heterologous functional regions. The fusion does not necessarily need to be direct, but may occur through linker sequences.

Moreover, fusion proteins may also be engineered to improve characteristics of  
30 the polypeptide of the present invention. For instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence during purification from the host cell or subsequent handling and storage. Also, peptide moieties may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final  
35 preparation of the polypeptide. The addition of peptide moieties to facilitate handling of polypeptides are familiar and routine techniques in the art.



Moreover, polypeptides of the present invention, including fragments, and specifically epitopes, can be combined with parts of the constant domain of immunoglobulins (IgG), resulting in chimeric polypeptides. These fusion proteins facilitate purification and show an increased half-life in vivo. One reported example describes chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. (EP A 394,827; Traunecker et al., *Nature* 331:84-86 (1988).) Fusion proteins having disulfide-linked dimeric structures (due to the IgG) can also be more efficient in binding and neutralizing other molecules, than the monomeric secreted protein or protein fragment alone. (Fountoulakis et al., *J. Biochem.* 270:3958-3964 (1995).)

Similarly, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties. (EP-A 0232 262.) Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified, would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. (See, D. Bennett et al., *J. Molecular Recognition* 8:52-58 (1995); K. Johanson et al., *J. Biol. Chem.* 270:9459-9471 (1995).)

Moreover, the polypeptides of the present invention can be fused to marker sequences, such as a peptide which facilitates purification of the fused polypeptide. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., *Proc. Natl. Acad. Sci. USA* 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Another peptide tag useful for purification, the "HA" tag, corresponds to an epitope derived from the influenza hemagglutinin protein. (Wilson et al., *Cell* 37:767 (1984).)

Thus, any of these above fusions can be engineered using the polynucleotides or the polypeptides of the present invention.

### Vectors, Host Cells, and Protein Production

The present invention also relates to vectors containing the polynucleotide of the present invention, host cells, and the production of polypeptides by recombinant techniques. The vector may be, for example, a phage, plasmid, viral, or retroviral  
5 vector. Retroviral vectors may be replication competent or replication defective. In the latter case, viral propagation generally will occur only in complementing host cells.

The polynucleotides may be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is  
10 a virus, it may be packaged in vitro using an appropriate packaging cell line and then transduced into host cells.

The polynucleotide insert should be operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the *E. coli* lac, trp, phoA and tac promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to  
15 name a few. Other suitable promoters will be known to the skilled artisan. The expression constructs will further contain sites for transcription initiation, termination, and, in the transcribed region, a ribosome binding site for translation. The coding portion of the transcripts expressed by the constructs will preferably include a translation initiating codon at the beginning and a termination codon (UAA, UGA or  
20 UAG) appropriately positioned at the end of the polypeptide to be translated.

As indicated, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase, G418 or neomycin resistance for eukaryotic cell culture and tetracycline, kanamycin or ampicillin resistance  
25 genes for culturing in *E. coli* and other bacteria. Representative examples of appropriate hosts include, but are not limited to, bacterial cells, such as *E. coli*, *Streptomyces* and *Salmonella typhimurium* cells; fungal cells, such as yeast cells; insect cells such as *Drosophila* S2 and *Spodoptera Sf9* cells; animal cells such as CHO, COS, 293, and Bowes melanoma cells; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art.

Among vectors preferred for use in bacteria include pQE70, pQE60 and pQE-9, available from QIAGEN, Inc.; pBluescript vectors, Phagescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, available from Stratagene Cloning Systems, Inc.; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia Biotech, Inc. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1  
35 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Other suitable vectors will be readily apparent to the skilled artisan.

Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection, or other methods. Such methods are described in many standard laboratory manuals, such as Davis et al., Basic Methods  
5 In Molecular Biology (1986). It is specifically contemplated that the polypeptides of the present invention may in fact be expressed by a host cell lacking a recombinant vector.

A polypeptide of this invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography,  
10 phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification.

Polypeptides of the present invention, and preferably the secreted form, can also  
15 be recovered from: products purified from natural sources, including bodily fluids, tissues and cells, whether directly isolated or cultured; products of chemical synthetic procedures; and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect, and mammalian cells. Depending upon the host employed in a recombinant production  
20 procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes. Thus, it is well known in the art that the N-terminal methionine encoded by the translation initiation codon generally is removed with high efficiency from any protein  
25 after translation in all eukaryotic cells. While the N-terminal methionine on most proteins also is efficiently removed in most prokaryotes, for some proteins, this prokaryotic removal process is inefficient, depending on the nature of the amino acid to which the N-terminal methionine is covalently linked.

### 30 Uses of the Polynucleotides

Each of the polynucleotides identified herein can be used in numerous ways as reagents. The following description should be considered exemplary and utilizes known techniques.

The polynucleotides of the present invention are useful for chromosome  
35 identification. There exists an ongoing need to identify new chromosome markers, since few chromosome marking reagents, based on actual sequence data (repeat

polymorphisms), are presently available. Each polynucleotide of the present invention can be used as a chromosome marker.

Briefly, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the sequences shown in SEQ ID NO:X. Primers can be  
5 selected using computer analysis so that primers do not span more than one predicted exon in the genomic DNA. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the SEQ ID NO:X will yield an amplified fragment.

Similarly, somatic hybrids provide a rapid method of PCR mapping the  
10 polynucleotides to particular chromosomes. Three or more clones can be assigned per day using a single thermal cycler. Moreover, sublocalization of the polynucleotides can be achieved with panels of specific chromosome fragments. Other gene mapping strategies that can be used include in situ hybridization, prescreening with labeled flow-sorted chromosomes, and preselection by hybridization to construct chromosome  
15 specific-cDNA libraries.

Precise chromosomal location of the polynucleotides can also be achieved using fluorescence in situ hybridization (FISH) of a metaphase chromosomal spread. This technique uses polynucleotides as short as 500 or 600 bases; however, polynucleotides 2,000-4,000 bp are preferred. For a review of this technique, see Verma et al.,  
20 "Human Chromosomes: a Manual of Basic Techniques," Pergamon Press, New York (1988).

For chromosome mapping, the polynucleotides can be used individually (to mark a single chromosome or a single site on that chromosome) or in panels (for marking multiple sites and/or multiple chromosomes). Preferred polynucleotides  
25 correspond to the noncoding regions of the cDNAs because the coding sequences are more likely conserved within gene families, thus increasing the chance of cross hybridization during chromosomal mapping.

Once a polynucleotide has been mapped to a precise chromosomal location, the physical position of the polynucleotide can be used in linkage analysis. Linkage  
30 analysis establishes coinheritance between a chromosomal location and presentation of a particular disease. (Disease mapping data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library) .) Assuming 1 megabase mapping resolution and one gene per 20 kb, a cDNA precisely localized to a chromosomal region associated with the disease  
35 could be one of 50-500 potential causative genes.

Thus, once coinheritance is established, differences in the polynucleotide and the corresponding gene between affected and unaffected individuals can be examined.

First, visible structural alterations in the chromosomes, such as deletions or translocations, are examined in chromosome spreads or by PCR. If no structural alterations exist, the presence of point mutations are ascertained. Mutations observed in some or all affected individuals, but not in normal individuals, indicates that the  
5 mutation may cause the disease. However, complete sequencing of the polypeptide and the corresponding gene from several normal individuals is required to distinguish the mutation from a polymorphism. If a new polymorphism is identified, this polymorphic polypeptide can be used for further linkage analysis.

Furthermore, increased or decreased expression of the gene in affected  
10 individuals as compared to unaffected individuals can be assessed using polynucleotides of the present invention. Any of these alterations (altered expression, chromosomal rearrangement, or mutation) can be used as a diagnostic or prognostic marker.

In addition to the foregoing, a polynucleotide can be used to control gene  
15 expression through triple helix formation or antisense DNA or RNA. Both methods rely on binding of the polynucleotide to DNA or RNA. For these techniques, preferred polynucleotides are usually 20 to 40 bases in length and complementary to either the region of the gene involved in transcription (triple helix - see Lee et al., Nucl. Acids Res. 6:3073 (1979); Cooney et al., Science 241:456 (1988); and Dervan et al., Science  
20 251:1360 (1991) ) or to the mRNA itself (antisense - Okano, J. Neurochem. 56:560 (1991); Oligodeoxy-nucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988).) Triple helix formation optimally results in a shut-off of RNA transcription from DNA, while antisense RNA hybridization blocks translation of an mRNA molecule into polypeptide. Both techniques are effective in model  
25 systems, and the information disclosed herein can be used to design antisense or triple helix polynucleotides in an effort to treat disease.

Polynucleotides of the present invention are also useful in gene therapy. One goal of gene therapy is to insert a normal gene into an organism having a defective gene, in an effort to correct the genetic defect. The polynucleotides disclosed in the  
30 present invention offer a means of targeting such genetic defects in a highly accurate manner. Another goal is to insert a new gene that was not present in the host genome, thereby producing a new trait in the host cell.

The polynucleotides are also useful for identifying individuals from minute biological samples. The United States military, for example, is considering the use of  
35 restriction fragment length polymorphism (RFLP) for identification of its personnel. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identifying

personnel. This method does not suffer from the current limitations of "Dog Tags" which can be lost, switched, or stolen, making positive identification difficult. The polynucleotides of the present invention can be used as additional DNA markers for RFLP.

5           The polynucleotides of the present invention can also be used as an alternative to RFLP, by determining the actual base-by-base DNA sequence of selected portions of an individual's genome. These sequences can be used to prepare PCR primers for amplifying and isolating such selected DNA, which can then be sequenced. Using this technique, individuals can be identified because each individual will have a unique set  
10 of DNA sequences. Once an unique ID database is established for an individual, positive identification of that individual, living or dead, can be made from extremely small tissue samples.

          Forensic biology also benefits from using DNA-based identification techniques as disclosed herein. DNA sequences taken from very small biological samples such as  
15 tissues, e.g., hair or skin, or body fluids, e.g., blood, saliva, semen, etc., can be amplified using PCR. In one prior art technique, gene sequences amplified from polymorphic loci, such as DQa class II HLA gene, are used in forensic biology to identify individuals. (Erlich, H., PCR Technology, Freeman and Co. (1992).) Once these specific polymorphic loci are amplified, they are digested with one or more  
20 restriction enzymes, yielding an identifying set of bands on a Southern blot probed with DNA corresponding to the DQa class II HLA gene. Similarly, polynucleotides of the present invention can be used as polymorphic markers for forensic purposes.

          There is also a need for reagents capable of identifying the source of a particular tissue. Such need arises, for example, in forensics when presented with tissue of  
25 unknown origin. Appropriate reagents can comprise, for example, DNA probes or primers specific to particular tissue prepared from the sequences of the present invention. Panels of such reagents can identify tissue by species and/or by organ type. In a similar fashion, these reagents can be used to screen tissue cultures for contamination.

30           In the very least, the polynucleotides of the present invention can be used as molecular weight markers on Southern gels, as diagnostic probes for the presence of a specific mRNA in a particular cell type, as a probe to "subtract-out" known sequences in the process of discovering novel polynucleotides, for selecting and making oligomers for attachment to a "gene chip" or other support, to raise anti-DNA antibodies using  
35 DNA immunization techniques, and as an antigen to elicit an immune response.

### Uses of the Polypeptides

Each of the polypeptides identified herein can be used in numerous ways. The following description should be considered exemplary and utilizes known techniques.

A polypeptide of the present invention can be used to assay protein levels in a biological sample using antibody-based techniques. For example, protein expression in tissues can be studied with classical immunohistological methods. (Jalkanen, M., et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, M., et al., J. Cell. Biol. 105:3087-3096 (1987).) Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase, and radioisotopes, such as iodine ( $^{125}\text{I}$ ,  $^{121}\text{I}$ ), carbon ( $^{14}\text{C}$ ), sulfur ( $^{35}\text{S}$ ), tritium ( $^3\text{H}$ ), indium ( $^{112}\text{In}$ ), and technetium ( $^{99\text{m}}\text{Tc}$ ), and fluorescent labels, such as fluorescein and rhodamine, and biotin.

In addition to assaying secreted protein levels in a biological sample, proteins can also be detected in vivo by imaging. Antibody labels or markers for in vivo imaging of protein include those detectable by X-radiography, NMR or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the antibody by labeling of nutrients for the relevant hybridoma.

A protein-specific antibody or antibody fragment which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example,  $^{131}\text{I}$ ,  $^{112}\text{In}$ ,  $^{99\text{m}}\text{Tc}$ ), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously, or intraperitoneally) into the mammal. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of  $^{99\text{m}}\text{Tc}$ . The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain the specific protein. In vivo tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments." (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982).)

Thus, the invention provides a diagnostic method of a disorder, which involves (a) assaying the expression of a polypeptide of the present invention in cells or body fluid of an individual; (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of a disorder.

Moreover, polypeptides of the present invention can be used to treat disease. For example, patients can be administered a polypeptide of the present invention in an effort to replace absent or decreased levels of the polypeptide (e.g., insulin), to supplement absent or decreased levels of a different polypeptide (e.g., hemoglobin S for hemoglobin B), to inhibit the activity of a polypeptide (e.g., an oncogene), to activate the activity of a polypeptide (e.g., by binding to a receptor), to reduce the activity of a membrane bound receptor by competing with it for free ligand (e.g., soluble TNF receptors used in reducing inflammation), or to bring about a desired response (e.g., blood vessel growth).

Similarly, antibodies directed to a polypeptide of the present invention can also be used to treat disease. For example, administration of an antibody directed to a polypeptide of the present invention can bind and reduce overproduction of the polypeptide. Similarly, administration of an antibody can activate the polypeptide, such as by binding to a polypeptide bound to a membrane (receptor).

At the very least, the polypeptides of the present invention can be used as molecular weight markers on SDS-PAGE gels or on molecular sieve gel filtration columns using methods well known to those of skill in the art. Polypeptides can also be used to raise antibodies, which in turn are used to measure protein expression from a recombinant cell, as a way of assessing transformation of the host cell. Moreover, the polypeptides of the present invention can be used to test the following biological activities.

### **Biological Activities**

The polynucleotides and polypeptides of the present invention can be used in assays to test for one or more biological activities. If these polynucleotides and polypeptides do exhibit activity in a particular assay, it is likely that these molecules may be involved in the diseases associated with the biological activity. Thus, the polynucleotides and polypeptides could be used to treat the associated disease.

### **Immune Activity**

A polypeptide or polynucleotide of the present invention may be useful in treating deficiencies or disorders of the immune system, by activating or inhibiting the



proliferation, differentiation, or mobilization (chemotaxis) of immune cells. Immune cells develop through a process called hematopoiesis, producing myeloid (platelets, red blood cells, neutrophils, and macrophages) and lymphoid (B and T lymphocytes) cells from pluripotent stem cells. The etiology of these immune deficiencies or disorders may be genetic, somatic, such as cancer or some autoimmune disorders, acquired (e.g., by chemotherapy or toxins), or infectious. Moreover, a polynucleotide or polypeptide of the present invention can be used as a marker or detector of a particular immune system disease or disorder.

A polynucleotide or polypeptide of the present invention may be useful in treating or detecting deficiencies or disorders of hematopoietic cells. A polypeptide or polynucleotide of the present invention could be used to increase differentiation and proliferation of hematopoietic cells, including the pluripotent stem cells, in an effort to treat those disorders associated with a decrease in certain (or many) types hematopoietic cells. Examples of immunologic deficiency syndromes include, but are not limited to: blood protein disorders (e.g. agammaglobulinemia, dysgammaglobulinemia), ataxia telangiectasia, common variable immunodeficiency, DiGeorge Syndrome, HIV infection, HTLV-BLV infection, leukocyte adhesion deficiency syndrome, lymphopenia, phagocyte bactericidal dysfunction, severe combined immunodeficiency (SCIDs), Wiskott-Aldrich Disorder, anemia, thrombocytopenia, or hemoglobinuria.

Moreover, a polypeptide or polynucleotide of the present invention could also be used to modulate hemostatic (the stopping of bleeding) or thrombolytic activity (clot formation). For example, by increasing hemostatic or thrombolytic activity, a polynucleotide or polypeptide of the present invention could be used to treat blood coagulation disorders (e.g., afibrinogenemia, factor deficiencies), blood platelet disorders (e.g. thrombocytopenia), or wounds resulting from trauma, surgery, or other causes. Alternatively, a polynucleotide or polypeptide of the present invention that can decrease hemostatic or thrombolytic activity could be used to inhibit or dissolve clotting. These molecules could be important in the treatment of heart attacks (infarction), strokes, or scarring.

A polynucleotide or polypeptide of the present invention may also be useful in treating or detecting autoimmune disorders. Many autoimmune disorders result from inappropriate recognition of self as foreign material by immune cells. This inappropriate recognition results in an immune response leading to the destruction of the host tissue. Therefore, the administration of a polypeptide or polynucleotide of the present invention that inhibits an immune response, particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing autoimmune disorders.

Examples of autoimmune disorders that can be treated or detected by the present invention include, but are not limited to: Addison's Disease, hemolytic anemia, antiphospholipid syndrome, rheumatoid arthritis, dermatitis, allergic encephalomyelitis, glomerulonephritis, Goodpasture's Syndrome, Graves' Disease, Multiple Sclerosis, Myasthenia Gravis, Neuritis, Ophthalmia, Bullous Pemphigoid, Pemphigus, Polyendocrinopathies, Purpura, Reiter's Disease, Stiff-Man Syndrome, Autoimmune Thyroiditis, Systemic Lupus Erythematosus, Autoimmune Pulmonary Inflammation, Guillain-Barre Syndrome, insulin dependent diabetes mellitus, and autoimmune inflammatory eye disease.

10 Similarly, allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems, may also be treated by a polypeptide or polynucleotide of the present invention. Moreover, these molecules can be used to treat anaphylaxis, hypersensitivity to an antigenic molecule, or blood group incompatibility.

A polynucleotide or polypeptide of the present invention may also be used to 15 treat and/or prevent organ rejection or graft-versus-host disease (GVHD). Organ rejection occurs by host immune cell destruction of the transplanted tissue through an immune response. Similarly, an immune response is also involved in GVHD, but, in this case, the foreign transplanted immune cells destroy the host tissues. The administration of a polypeptide or polynucleotide of the present invention that inhibits 20 an immune response, particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing organ rejection or GVHD.

Similarly, a polypeptide or polynucleotide of the present invention may also be used to modulate inflammation. For example, the polypeptide or polynucleotide may inhibit the proliferation and differentiation of cells involved in an inflammatory 25 response. These molecules can be used to treat inflammatory conditions, both chronic and acute conditions, including inflammation associated with infection (e.g., septic shock, sepsis, or systemic inflammatory response syndrome (SIRS)), ischemia-reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine induced lung injury, inflammatory bowel 30 disease, Crohn's disease, or resulting from over production of cytokines (e.g., TNF or IL-1.)

#### **Hyperproliferative Disorders**

A polypeptide or polynucleotide can be used to treat or detect hyperproliferative 35 disorders, including neoplasms. A polypeptide or polynucleotide of the present invention may inhibit the proliferation of the disorder through direct or indirect

interactions. Alternatively, a polypeptide or polynucleotide of the present invention may proliferate other cells which can inhibit the hyperproliferative disorder.

For example, by increasing an immune response, particularly increasing antigenic qualities of the hyperproliferative disorder or by proliferating, differentiating, or mobilizing T-cells, hyperproliferative disorders can be treated. This immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, decreasing an immune response may also be a method of treating hyperproliferative disorders, such as a chemotherapeutic agent.

Examples of hyperproliferative disorders that can be treated or detected by a polynucleotide or polypeptide of the present invention include, but are not limited to neoplasms located in the: abdomen, bone, breast, digestive system, liver, pancreas, peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous (central and peripheral), lymphatic system, pelvic, skin, soft tissue, spleen, thoracic, and urogenital.

Similarly, other hyperproliferative disorders can also be treated or detected by a polynucleotide or polypeptide of the present invention. Examples of such hyperproliferative disorders include, but are not limited to: hypergammaglobulinemia, lymphoproliferative disorders, paraproteinemias, purpura, sarcoidosis, Sezary Syndrome, Waldenstrom's Macroglobulinemia, Gaucher's Disease, histiocytosis, and any other hyperproliferative disease, besides neoplasia, located in an organ system listed above.

### **Infectious Disease**

A polypeptide or polynucleotide of the present invention can be used to treat or detect infectious agents. For example, by increasing the immune response, particularly increasing the proliferation and differentiation of B and/or T cells, infectious diseases may be treated. The immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, the polypeptide or polynucleotide of the present invention may also directly inhibit the infectious agent, without necessarily eliciting an immune response.

Viruses are one example of an infectious agent that can cause disease or symptoms that can be treated or detected by a polynucleotide or polypeptide of the present invention. Examples of viruses, include, but are not limited to the following DNA and RNA viral families: Arbovirus, Adenoviridae, Arenaviridae, Arterivirus, Bimaviridae, Bunyaviridae, Caliciviridae, Circoviridae, Coronaviridae, Flaviviridae, Hepadnaviridae (Hepatitis), Herpesviridae (such as, Cytomegalovirus, Herpes

- Simplex, Herpes Zoster), Mononegavirus (e.g., Paramyxoviridae, Morbillivirus, Rhabdoviridae), Orthomyxoviridae (e.g., Influenza), Papovaviridae, Parvoviridae, Picornaviridae, Poxviridae (such as Smallpox or Vaccinia), Reoviridae (e.g., Rotavirus), Retroviridae (HTLV-I, HTLV-II, Lentivirus), and Togaviridae (e.g., Rubivirus). Viruses falling within these families can cause a variety of diseases or symptoms, including, but not limited to: arthritis, bronchiolitis, encephalitis, eye infections (e.g., conjunctivitis, keratitis), chronic fatigue syndrome, hepatitis (A, B, C, E, Chronic Active, Delta), meningitis, opportunistic infections (e.g., AIDS), pneumonia, Burkitt's Lymphoma, chickenpox, hemorrhagic fever, Measles, Mumps, Parainfluenza, Rabies, the common cold, Polio, leukemia, Rubella, sexually transmitted diseases, skin diseases (e.g., Kaposi's, warts), and viremia. A polypeptide or polynucleotide of the present invention can be used to treat or detect any of these symptoms or diseases.

- Similarly, bacterial or fungal agents that can cause disease or symptoms and that can be treated or detected by a polynucleotide or polypeptide of the present invention include, but not limited to, the following Gram-Negative and Gram-positive bacterial families and fungi: Actinomycetales (e.g., Corynebacterium, Mycobacterium, Norcardia), Aspergillosis, Bacillaceae (e.g., Anthrax, Clostridium), Bacteroidaceae, Blastomycosis, Bordetella, Borrelia, Brucellosis, Candidiasis, Campylobacter, Coccidioidomycosis, Cryptococcosis, Dermatocycoses, Enterobacteriaceae (Klebsiella, Salmonella, Serratia, Yersinia), Erysipelothrix, Helicobacter, Legionellosis, Leptospirosis, Listeria, Mycoplasmatales, Neisseriaceae (e.g., Acinetobacter, Gonorrhea, Meningococcal), Pasteurellaceae Infections (e.g., Actinobacillus, Haemophilus, Pasteurella), Pseudomonas, Rickettsiaceae, Chlamydiaceae, Syphilis, and Staphylococcal. These bacterial or fungal families can cause the following diseases or symptoms, including, but not limited to: bacteremia, endocarditis, eye infections (conjunctivitis, tuberculosis, uveitis), gingivitis, opportunistic infections (e.g., AIDS related infections), paronychia, prosthesis-related infections, Reiter's Disease, respiratory tract infections, such as Whooping Cough or Empyema, sepsis, Lyme Disease, Cat-Scratch Disease, Dysentery, Paratyphoid Fever, food poisoning, Typhoid, pneumonia, Gonorrhea, meningitis, Chlamydia, Syphilis, Diphtheria, Leprosy, Paratuberculosis, Tuberculosis, Lupus, Botulism, gangrene, tetanus, impetigo, Rheumatic Fever, Scarlet Fever, sexually transmitted diseases, skin diseases (e.g., cellulitis, dermatocycoses), toxemia, urinary tract infections, wound infections. A polypeptide or polynucleotide of the present invention can be used to treat or detect any of these symptoms or diseases.

Moreover, parasitic agents causing disease or symptoms that can be treated or detected by a polynucleotide or polypeptide of the present invention include, but not limited to, the following families: Amebiasis, Babesiosis, Coccidiosis, Cryptosporidiosis, Dientamoebiasis, Dourine, Ectoparasitic, Giardiasis, Helminthiasis, Leishmaniasis, Theileriasis, Toxoplasmosis, Trypanosomiasis, and Trichomonas. These parasites can cause a variety of diseases or symptoms, including, but not limited to: Scabies, Trombiculiasis, eye infections, intestinal disease (e.g., dysentery, giardiasis), liver disease, lung disease, opportunistic infections (e.g., AIDS related), Malaria, pregnancy complications, and toxoplasmosis. A polypeptide or polynucleotide of the present invention can be used to treat or detect any of these symptoms or diseases.

Preferably, treatment using a polypeptide or polynucleotide of the present invention could either be by administering an effective amount of a polypeptide to the patient, or by removing cells from the patient, supplying the cells with a polynucleotide of the present invention, and returning the engineered cells to the patient (ex vivo therapy). Moreover, the polypeptide or polynucleotide of the present invention can be used as an antigen in a vaccine to raise an immune response against infectious disease.

### **Regeneration**

A polynucleotide or polypeptide of the present invention can be used to differentiate, proliferate, and attract cells, leading to the regeneration of tissues. (See, Science 276:59-87 (1997).) The regeneration of tissues could be used to repair, replace, or protect tissue damaged by congenital defects, trauma (wounds, burns, incisions, or ulcers), age, disease (e.g. osteoporosis, osteoarthritis, periodontal disease, liver failure), surgery, including cosmetic plastic surgery, fibrosis, reperfusion injury, or systemic cytokine damage.

Tissues that could be regenerated using the present invention include organs (e.g., pancreas, liver, intestine, kidney, skin, endothelium), muscle (smooth, skeletal or cardiac), vascular (including vascular endothelium), nervous, hematopoietic, and skeletal (bone, cartilage, tendon, and ligament) tissue. Preferably, regeneration occurs without or decreased scarring. Regeneration also may include angiogenesis.

Moreover, a polynucleotide or polypeptide of the present invention may increase regeneration of tissues difficult to heal. For example, increased tendon/ligament regeneration would quicken recovery time after damage. A polynucleotide or polypeptide of the present invention could also be used prophylactically in an effort to avoid damage. Specific diseases that could be treated include of tendinitis, carpal tunnel syndrome, and other tendon or ligament defects. A further example of tissue

regeneration of non-healing wounds includes pressure ulcers, ulcers associated with vascular insufficiency, surgical, and traumatic wounds.

Similarly, nerve and brain tissue could also be regenerated by using a polynucleotide or polypeptide of the present invention to proliferate and differentiate nerve cells. Diseases that could be treated using this method include central and peripheral nervous system diseases, neuropathies, or mechanical and traumatic disorders (e.g., spinal cord disorders, head trauma, cerebrovascular disease, and stroke). Specifically, diseases associated with peripheral nerve injuries, peripheral neuropathy (e.g., resulting from chemotherapy or other medical therapies), localized neuropathies, and central nervous system diseases (e.g., Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome), could all be treated using the polynucleotide or polypeptide of the present invention.

#### 15 **Chemotaxis**

A polynucleotide or polypeptide of the present invention may have chemotaxis activity. A chemotactic molecule attracts or mobilizes cells (e.g., monocytes, fibroblasts, neutrophils, T-cells, mast cells, eosinophils, epithelial and/or endothelial cells) to a particular site in the body, such as inflammation, infection, or site of hyperproliferation. The mobilized cells can then fight off and/or heal the particular trauma or abnormality.

A polynucleotide or polypeptide of the present invention may increase chemotactic activity of particular cells. These chemotactic molecules can then be used to treat inflammation, infection, hyperproliferative disorders, or any immune system disorder by increasing the number of cells targeted to a particular location in the body. For example, chemotactic molecules can be used to treat wounds and other trauma to tissues by attracting immune cells to the injured location. Chemotactic molecules of the present invention can also attract fibroblasts, which can be used to treat wounds.

It is also contemplated that a polynucleotide or polypeptide of the present invention may inhibit chemotactic activity. These molecules could also be used to treat disorders. Thus, a polynucleotide or polypeptide of the present invention could be used as an inhibitor of chemotaxis.

#### **Binding Activity**

A polypeptide of the present invention may be used to screen for molecules that bind to the polypeptide or for molecules to which the polypeptide binds. The binding of the polypeptide and the molecule may activate (agonist), increase, inhibit

(antagonist), or decrease activity of the polypeptide or the molecule bound. Examples of such molecules include antibodies, oligonucleotides, proteins (e.g., receptors), or small molecules.

Preferably, the molecule is closely related to the natural ligand of the polypeptide, e.g., a fragment of the ligand, or a natural substrate, a ligand, a structural or functional mimetic. (See, Coligan et al., Current Protocols in Immunology 1(2):Chapter 5 (1991).) Similarly, the molecule can be closely related to the natural receptor to which the polypeptide binds, or at least, a fragment of the receptor capable of being bound by the polypeptide (e.g., active site). In either case, the molecule can be rationally designed using known techniques.

Preferably, the screening for these molecules involves producing appropriate cells which express the polypeptide, either as a secreted protein or on the cell membrane. Preferred cells include cells from mammals, yeast, *Drosophila*, or *E. coli*. Cells expressing the polypeptide (or cell membrane containing the expressed polypeptide) are then preferably contacted with a test compound potentially containing the molecule to observe binding, stimulation, or inhibition of activity of either the polypeptide or the molecule.

The assay may simply test binding of a candidate compound to the polypeptide, wherein binding is detected by a label, or in an assay involving competition with a labeled competitor. Further, the assay may test whether the candidate compound results in a signal generated by binding to the polypeptide.

Alternatively, the assay can be carried out using cell-free preparations, polypeptide/molecule affixed to a solid support, chemical libraries, or natural product mixtures. The assay may also simply comprise the steps of mixing a candidate compound with a solution containing a polypeptide, measuring polypeptide/molecule activity or binding, and comparing the polypeptide/molecule activity or binding to a standard.

Preferably, an ELISA assay can measure polypeptide level or activity in a sample (e.g., biological sample) using a monoclonal or polyclonal antibody. The antibody can measure polypeptide level or activity by either binding, directly or indirectly, to the polypeptide or by competing with the polypeptide for a substrate.

All of these above assays can be used as diagnostic or prognostic markers. The molecules discovered using these assays can be used to treat disease or to bring about a particular result in a patient (e.g., blood vessel growth) by activating or inhibiting the polypeptide/molecule. Moreover, the assays can discover agents which may inhibit or enhance the production of the polypeptide from suitably manipulated cells or tissues.

Therefore, the invention includes a method of identifying compounds which bind to a polypeptide of the invention comprising the steps of: (a) incubating a candidate binding compound with a polypeptide of the invention; and (b) determining if binding has occurred. Moreover, the invention includes a method of identifying  
5 agonists/antagonists comprising the steps of: (a) incubating a candidate compound with a polypeptide of the invention, (b) assaying a biological activity, and (b) determining if a biological activity of the polypeptide has been altered.

### Other Activities

10 A polypeptide or polynucleotide of the present invention may also increase or decrease the differentiation or proliferation of embryonic stem cells, besides, as discussed above, hematopoietic lineage.

A polypeptide or polynucleotide of the present invention may also be used to modulate mammalian characteristics, such as body height, weight, hair color, eye color,  
15 skin, percentage of adipose tissue, pigmentation, size, and shape (e.g., cosmetic surgery). Similarly, a polypeptide or polynucleotide of the present invention may be used to modulate mammalian metabolism affecting catabolism, anabolism, processing, utilization, and storage of energy.

A polypeptide or polynucleotide of the present invention may be used to change  
20 a mammal's mental state or physical state by influencing biorhythms, circadian rhythms, depression (including depressive disorders), tendency for violence, tolerance for pain, reproductive capabilities (preferably by Activin or Inhibin-like activity), hormonal or endocrine levels, appetite, libido, memory, stress, or other cognitive qualities.

25 A polypeptide or polynucleotide of the present invention may also be used as a food additive or preservative, such as to increase or decrease storage capabilities, fat content, lipid, protein, carbohydrate, vitamins, minerals, cofactors or other nutritional components.

### Other Preferred Embodiments

Other preferred embodiments of the claimed invention include an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 50 contiguous nucleotides in the nucleotide sequence of  
35 SEQ ID NO:X wherein X is any integer as defined in Table 1.

Also preferred is a nucleic acid molecule wherein said sequence of contiguous nucleotides is included in the nucleotide sequence of SEQ ID NO:X in the range of



positions beginning with the nucleotide at about the position of the 5' Nucleotide of the Clone Sequence and ending with the nucleotide at about the position of the 3' Nucleotide of the Clone Sequence as defined for SEQ ID NO:X in Table 1.

Also preferred is a nucleic acid molecule wherein said sequence of contiguous  
5 nucleotides is included in the nucleotide sequence of SEQ ID NO:X in the range of positions beginning with the nucleotide at about the position of the 5' Nucleotide of the Start Codon and ending with the nucleotide at about the position of the 3' Nucleotide of the Clone Sequence as defined for SEQ ID NO:X in Table 1.

Similarly preferred is a nucleic acid molecule wherein said sequence of  
10 contiguous nucleotides is included in the nucleotide sequence of SEQ ID NO:X in the range of positions beginning with the nucleotide at about the position of the 5' Nucleotide of the First Amino Acid of the Signal Peptide and ending with the nucleotide at about the position of the 3' Nucleotide of the Clone Sequence as defined for SEQ ID NO:X in Table 1.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide  
15 sequence which is at least 95% identical to a sequence of at least about 150 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X.

Further preferred is an isolated nucleic acid molecule comprising a nucleotide  
sequence which is at least 95% identical to a sequence of at least about 500 contiguous  
20 nucleotides in the nucleotide sequence of SEQ ID NO:X.

A further preferred embodiment is a nucleic acid molecule comprising a  
nucleotide sequence which is at least 95% identical to the nucleotide sequence of SEQ  
ID NO:X beginning with the nucleotide at about the position of the 5' Nucleotide of the  
First Amino Acid of the Signal Peptide and ending with the nucleotide at about the  
25 position of the 3' Nucleotide of the Clone Sequence as defined for SEQ ID NO:X in  
Table 1.

A further preferred embodiment is an isolated nucleic acid molecule comprising  
a nucleotide sequence which is at least 95% identical to the complete nucleotide  
sequence of SEQ ID NO:X.

Also preferred is an isolated nucleic acid molecule which hybridizes under  
30 stringent hybridization conditions to a nucleic acid molecule, wherein said nucleic acid molecule which hybridizes does not hybridize under stringent hybridization conditions to a nucleic acid molecule having a nucleotide sequence consisting of only A residues or of only T residues.

Also preferred is a composition of matter comprising a DNA molecule which  
35 comprises a human cDNA clone identified by a cDNA Clone Identifier in Table 1,  
which DNA molecule is contained in the material deposited with the American Type

Culture Collection and given the ATCC Deposit Number shown in Table 1 for said cDNA Clone Identifier.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least 50 contiguous nucleotides in the nucleotide sequence of a human cDNA clone identified by a cDNA Clone Identifier in Table 1, which DNA molecule is contained in the deposit given the ATCC Deposit Number shown in Table 1.

Also preferred is an isolated nucleic acid molecule, wherein said sequence of at least 50 contiguous nucleotides is included in the nucleotide sequence of the complete open reading frame sequence encoded by said human cDNA clone.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to sequence of at least 150 contiguous nucleotides in the nucleotide sequence encoded by said human cDNA clone.

A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to sequence of at least 500 contiguous nucleotides in the nucleotide sequence encoded by said human cDNA clone.

A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the complete nucleotide sequence encoded by said human cDNA clone.

A further preferred embodiment is a method for detecting in a biological sample a nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1; and a nucleotide sequence encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1; which method comprises a step of comparing a nucleotide sequence of at least one nucleic acid molecule in said sample with a sequence selected from said group and determining whether the sequence of said nucleic acid molecule in said sample is at least 95% identical to said selected sequence.

Also preferred is the above method wherein said step of comparing sequences comprises determining the extent of nucleic acid hybridization between nucleic acid molecules in said sample and a nucleic acid molecule comprising said sequence selected from said group. Similarly, also preferred is the above method wherein said step of comparing sequences is performed by comparing the nucleotide sequence determined from a nucleic acid molecule in said sample with said sequence selected from said group. The nucleic acid molecules can comprise DNA molecules or RNA molecules.

A further preferred embodiment is a method for identifying the species, tissue or cell type of a biological sample which method comprises a step of detecting nucleic acid molecules in said sample, if any, comprising a nucleotide sequence that is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1; and a nucleotide sequence encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

The method for identifying the species, tissue or cell type of a biological sample can comprise a step of detecting nucleic acid molecules comprising a nucleotide sequence in a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from said group.

Also preferred is a method for diagnosing in a subject a pathological condition associated with abnormal structure or expression of a gene encoding a secreted protein identified in Table 1, which method comprises a step of detecting in a biological sample obtained from said subject nucleic acid molecules, if any, comprising a nucleotide sequence that is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1; and a nucleotide sequence encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

The method for diagnosing a pathological condition can comprise a step of detecting nucleic acid molecules comprising a nucleotide sequence in a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from said group.

Also preferred is a composition of matter comprising isolated nucleic acid molecules wherein the nucleotide sequences of said nucleic acid molecules comprise a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1; and a nucleotide sequence encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1. The nucleic acid molecules can comprise DNA molecules or RNA molecules.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 90% identical to a sequence of at least about 10 contiguous amino acids in the amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1.

Also preferred is a polypeptide, wherein said sequence of contiguous amino acids is included in the amino acid sequence of SEQ ID NO:Y in the range of positions beginning with the residue at about the position of the First Amino Acid of the Secreted Portion and ending with the residue at about the Last Amino Acid of the Open Reading Frame as set forth for SEQ ID NO:Y in Table 1.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 30 contiguous amino acids in the amino acid sequence of SEQ ID NO:Y.

Further preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 100 contiguous amino acids in the amino acid sequence of SEQ ID NO:Y.

Further preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to the complete amino acid sequence of SEQ ID NO:Y.

Further preferred is an isolated polypeptide comprising an amino acid sequence at least 90% identical to a sequence of at least about 10 contiguous amino acids in the complete amino acid sequence of a secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is a polypeptide wherein said sequence of contiguous amino acids is included in the amino acid sequence of a secreted portion of the secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 30 contiguous amino acids in the amino acid sequence of the secreted portion of the protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 100 contiguous amino acids in the amino acid sequence of the secreted portion of the protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to the amino acid sequence of the secreted portion of the protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Further preferred is an isolated antibody which binds specifically to a polypeptide comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Further preferred is a method for detecting in a biological sample a polypeptide comprising an amino acid sequence which is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1; which method comprises a step of comparing an amino acid sequence of at least one polypeptide molecule in said sample with a sequence selected from said group and determining whether the sequence of said polypeptide molecule in said sample is at least 90% identical to said sequence of at least 10 contiguous amino acids.

Also preferred is the above method wherein said step of comparing an amino acid sequence of at least one polypeptide molecule in said sample with a sequence selected from said group comprises determining the extent of specific binding of polypeptides in said sample to an antibody which binds specifically to a polypeptide comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is the above method wherein said step of comparing sequences is performed by comparing the amino acid sequence determined from a polypeptide molecule in said sample with said sequence selected from said group.

Also preferred is a method for identifying the species, tissue or cell type of a biological sample which method comprises a step of detecting polypeptide molecules in said sample, if any, comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as  
5 defined in Table 1; and a complete amino acid sequence of a secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is the above method for identifying the species, tissue or cell type  
10 of a biological sample, which method comprises a step of detecting polypeptide molecules comprising an amino acid sequence in a panel of at least two amino acid sequences, wherein at least one sequence in said panel is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the above group.

Also preferred is a method for diagnosing in a subject a pathological condition associated with abnormal structure or expression of a gene encoding a secreted protein identified in Table 1, which method comprises a step of detecting in a biological sample obtained from said subject polypeptide molecules comprising an amino acid sequence in a panel of at least two amino acid sequences, wherein at least one sequence in said panel  
20 is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number  
25 shown for said cDNA clone in Table 1.

In any of these methods, the step of detecting said polypeptide molecules includes using an antibody.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a nucleotide sequence encoding a  
30 polypeptide wherein said polypeptide comprises an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table  
35 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is an isolated nucleic acid molecule, wherein said nucleotide sequence encoding a polypeptide has been optimized for expression of said polypeptide in a prokaryotic host.

Also preferred is an isolated nucleic acid molecule, wherein said polypeptide  
5 comprises an amino acid sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

10 Further preferred is a method of making a recombinant vector comprising inserting any of the above isolated nucleic acid molecule into a vector. Also preferred is the recombinant vector produced by this method. Also preferred is a method of making a recombinant host cell comprising introducing the vector into a host cell, as well as the recombinant host cell produced by this method.

15 Also preferred is a method of making an isolated polypeptide comprising culturing this recombinant host cell under conditions such that said polypeptide is expressed and recovering said polypeptide. Also preferred is this method of making an isolated polypeptide, wherein said recombinant host cell is a eukaryotic cell and said polypeptide is a secreted portion of a human secreted protein comprising an amino acid  
20 sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y beginning with the residue at the position of the First Amino Acid of the Secreted Portion of SEQ ID NO:Y wherein Y is an integer set forth in Table 1 and said position of the First Amino Acid of the Secreted Portion of SEQ ID NO:Y is defined in Table 1; and an amino acid sequence of a secreted portion of a protein encoded by a human  
25 cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1. The isolated polypeptide produced by this method is also preferred.

Also preferred is a method of treatment of an individual in need of an increased level of a secreted protein activity, which method comprises administering to such an  
30 individual a pharmaceutical composition comprising an amount of an isolated polypeptide, polynucleotide, or antibody of the claimed invention effective to increase the level of said protein activity in said individual.

Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of  
35 illustration and are not intended as limiting.

### Examples

#### Example 1: Isolation of a Selected cDNA Clone From the Deposited Sample

5 Each cDNA clone in a cited ATCC deposit is contained in a plasmid vector. Table 1 identifies the vectors used to construct the cDNA library from which each clone was isolated. In many cases, the vector used to construct the library is a phage vector from which a plasmid has been excised. The table immediately below correlates the related plasmid for each phage vector used in constructing the cDNA library. For  
10 example, where a particular clone is identified in Table 1 as being isolated in the vector "Lambda Zap," the corresponding deposited clone is in "pBluescript."

	<u>Vector Used to Construct Library</u>	<u>Corresponding Deposited Plasmid</u>
	Lambda Zap	pBluescript (pBS)
	Uni-Zap XR	pBluescript (pBS)
15	Zap Express	pBK
	lafmid BA	plafmid BA
	pSport1	pSport1
	pCMVSPORT 2.0	pCMVSPORT 2.0
	pCMVSPORT 3.0	pCMVSPORT 3.0
20	pCR <sup>®</sup> 2.1	pCR <sup>®</sup> 2.1

Vectors Lambda Zap (U.S. Patent Nos. 5,128,256 and 5,286,636), Uni-Zap XR (U.S. Patent Nos. 5,128, 256 and 5,286,636), Zap Express (U.S. Patent Nos. 5,128,256 and 5,286,636), pBluescript (pBS) (Short, J. M. et al., Nucleic Acids Res. 16:7583-7600 (1988); Alting-Mees, M. A. and Short, J. M., Nucleic Acids Res. 25 17:9494 (1989)) and pBK (Alting-Mees, M. A. et al., Strategies 5:58-61 (1992)) are commercially available from Stratagene Cloning Systems, Inc., 11011 N. Torrey Pines Road, La Jolla, CA, 92037. pBS contains an ampicillin resistance gene and pBK contains a neomycin resistance gene. Both can be transformed into E. coli strain XL-1 Blue, also available from Stratagene. pBS comes in 4 forms SK+, SK-, KS+ and KS. The S and K refers to the orientation of the polylinker to the T7 and T3 primer  
30 sequences which flank the polylinker region ("S" is for SacI and "K" is for KpnI which are the first sites on each respective end of the linker). "+" or "-" refer to the orientation of the fl origin of replication ("ori"), such that in one orientation, single stranded rescue initiated from the fl ori generates sense strand DNA and in the other, antisense.

35 Vectors pSport1, pCMVSPORT 2.0 and pCMVSPORT 3.0, were obtained from Life Technologies, Inc., P. O. Box 6009, Gaithersburg, MD 20897. All Sport vectors contain an ampicillin resistance gene and may be transformed into E. coli strain



DH10B, also available from Life Technologies. (See, for instance, Gruber, C. E., et al., *Focus* 15:59 (1993).) Vector lafmid BA (Bento Soares, Columbia University, NY) contains an ampicillin resistance gene and can be transformed into E. coli strain XL-1 Blue. Vector pCR<sup>®</sup>2.1, which is available from Invitrogen, 1600 Faraday Avenue,  
5 Carlsbad, CA 92008, contains an ampicillin resistance gene and may be transformed into E. coli strain DH10B, available from Life Technologies. (See, for instance, Clark, J. M., *Nuc. Acids Res.* 16:9677-9686 (1988) and Mead, D. et al., *Bio/Technology* 9: (1991).) Preferably, a polynucleotide of the present invention does not comprise the phage vector sequences identified for the particular clone in Table 1, as well as the  
10 corresponding plasmid vector sequences designated above.

The deposited material in the sample assigned the ATCC Deposit Number cited in Table 1 for any given cDNA clone also may contain one or more additional plasmids, each comprising a cDNA clone different from that given clone. Thus, deposits sharing the same ATCC Deposit Number contain at least a plasmid for each cDNA clone  
15 identified in Table 1. Typically, each ATCC deposit sample cited in Table 1 comprises a mixture of approximately equal amounts (by weight) of about 50 plasmid DNAs, each containing a different cDNA clone; but such a deposit sample may include plasmids for more or less than 50 cDNA clones, up to about 500 cDNA clones.

Two approaches can be used to isolate a particular clone from the deposited  
20 sample of plasmid DNAs cited for that clone in Table 1. First, a plasmid is directly isolated by screening the clones using a polynucleotide probe corresponding to SEQ ID NO:X.

Particularly, a specific polynucleotide with 30-40 nucleotides is synthesized using an Applied Biosystems DNA synthesizer according to the sequence reported.  
25 The oligonucleotide is labeled, for instance, with <sup>32</sup>P-γ-ATP using T4 polynucleotide kinase and purified according to routine methods. (E.g., Maniatis et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press, Cold Spring, NY (1982).) The plasmid mixture is transformed into a suitable host, as indicated above (such as XL-1 Blue (Stratagene)) using techniques known to those of skill in the art, such as  
30 those provided by the vector supplier or in related publications or patents cited above. The transformants are plated on 1.5% agar plates (containing the appropriate selection agent, e.g., ampicillin) to a density of about 150 transformants (colonies) per plate. These plates are screened using Nylon membranes according to routine methods for bacterial colony screening (e.g., Sambrook et al., *Molecular Cloning: A Laboratory*  
35 *Manual*, 2nd Edit., (1989), Cold Spring Harbor Laboratory Press, pages 1.93 to 1.104), or other techniques known to those of skill in the art.

Alternatively, two primers of 17-20 nucleotides derived from both ends of the SEQ ID NO:X (i.e., within the region of SEQ ID NO:X bounded by the 5' NT and the 3' NT of the clone defined in Table 1) are synthesized and used to amplify the desired cDNA using the deposited cDNA plasmid as a template. The polymerase chain reaction is carried out under routine conditions, for instance, in 25  $\mu$ l of reaction mixture with 0.5  $\mu$ g of the above cDNA template. A convenient reaction mixture is 1.5-5 mM  $\text{MgCl}_2$ , 0.01 % (w/v) gelatin, 20  $\mu$ M each of dATP, dCTP, dGTP, dTTP, 25 pmol of each primer and 0.25 Unit of Taq polymerase. Thirty five cycles of PCR (denaturation at 94°C for 1 min; annealing at 55°C for 1 min; elongation at 72°C for 1 min) are performed with a Perkin-Elmer Cetus automated thermal cycler. The amplified product is analyzed by agarose gel electrophoresis and the DNA band with expected molecular weight is excised and purified. The PCR product is verified to be the selected sequence by subcloning and sequencing the DNA product.

Several methods are available for the identification of the 5' or 3' non-coding portions of a gene which may not be present in the deposited clone. These methods include but are not limited to, filter probing, clone enrichment using specific probes, and protocols similar or identical to 5' and 3' "RACE" protocols which are well known in the art. For instance, a method similar to 5' RACE is available for generating the missing 5' end of a desired full-length transcript. (Fromont-Racine et al., Nucleic Acids Res. 21(7):1683-1684 (1993).)

Briefly, a specific RNA oligonucleotide is ligated to the 5' ends of a population of RNA presumably containing full-length gene RNA transcripts. A primer set containing a primer specific to the ligated RNA oligonucleotide and a primer specific to a known sequence of the gene of interest is used to PCR amplify the 5' portion of the desired full-length gene. This amplified product may then be sequenced and used to generate the full length gene.

This above method starts with total RNA isolated from the desired source, although poly-A+ RNA can be used. The RNA preparation can then be treated with phosphatase if necessary to eliminate 5' phosphate groups on degraded or damaged RNA which may interfere with the later RNA ligase step. The phosphatase should then be inactivated and the RNA treated with tobacco acid pyrophosphatase in order to remove the cap structure present at the 5' ends of messenger RNAs. This reaction leaves a 5' phosphate group at the 5' end of the cap cleaved RNA which can then be ligated to an RNA oligonucleotide using T4 RNA ligase.

This modified RNA preparation is used as a template for first strand cDNA synthesis using a gene specific oligonucleotide. The first strand synthesis reaction is

used as a template for PCR amplification of the desired 5' end using a primer specific to the ligated RNA oligonucleotide and a primer specific to the known sequence of the gene of interest. The resultant product is then sequenced and analyzed to confirm that the 5' end sequence belongs to the desired gene.

5

**Example 2: Isolation of Genomic Clones Corresponding to a Polynucleotide**

A human genomic P1 library (Genomic Systems, Inc.) is screened by PCR using primers selected for the cDNA sequence corresponding to SEQ ID NO:X., according to the method described in Example 1. (See also, Sambrook.)

10

**Example 3: Tissue Distribution of Polypeptide**

Tissue distribution of mRNA expression of polynucleotides of the present invention is determined using protocols for Northern blot analysis, described by, among others, Sambrook et al. For example, a cDNA probe produced by the method described in Example 1 is labeled with P<sup>32</sup> using the rediprime™ DNA labeling system (Amersham Life Science), according to manufacturer's instructions. After labeling, the probe is purified using CHROMA SPIN-100™ column (Clontech Laboratories, Inc.), according to manufacturer's protocol number PT1200-1. The purified labeled probe is then used to examine various human tissues for mRNA expression.

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Multiple Tissue Northern (MTN) blots containing various human tissues (H) or human immune system tissues (IM) (Clontech) are examined with the labeled probe using ExpressHyb™ hybridization solution (Clontech) according to manufacturer's protocol number PT1190-1. Following hybridization and washing, the blots are mounted and exposed to film at -70°C overnight, and the films developed according to standard procedures.

25

**Example 4: Chromosomal Mapping of the Polynucleotides**

An oligonucleotide primer set is designed according to the sequence at the 5' end of SEQ ID NO:X. This primer preferably spans about 100 nucleotides. This primer set is then used in a polymerase chain reaction under the following set of conditions : 30 seconds, 95°C; 1 minute, 56°C; 1 minute, 70°C. This cycle is repeated 32 times followed by one 5 minute cycle at 70°C. Human, mouse, and hamster DNA is used as template in addition to a somatic cell hybrid panel containing individual chromosomes or chromosome fragments (Bios, Inc). The reactions is analyzed on

30

35

either 8% polyacrylamide gels or 3.5 % agarose gels. Chromosome mapping is determined by the presence of an approximately 100 bp PCR fragment in the particular somatic cell hybrid.

5 **Example 5: Bacterial Expression of a Polypeptide**

A polynucleotide encoding a polypeptide of the present invention is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' ends of the DNA sequence, as outlined in Example 1, to synthesize insertion fragments. The primers used to amplify the cDNA insert should preferably contain restriction sites, such as  
10 BamHI and XbaI, at the 5' end of the primers in order to clone the amplified product into the expression vector. For example, BamHI and XbaI correspond to the restriction enzyme sites on the bacterial expression vector pQE-9. (Qiagen, Inc., Chatsworth, CA). This plasmid vector encodes antibiotic resistance (Amp<sup>r</sup>), a bacterial origin of replication (ori), an IPTG-regulatable promoter/operator (P/O), a ribosome binding site  
15 (RBS), a 6-histidine tag (6-His), and restriction enzyme cloning sites.

The pQE-9 vector is digested with BamHI and XbaI and the amplified fragment is ligated into the pQE-9 vector maintaining the reading frame initiated at the bacterial RBS. The ligation mixture is then used to transform the E. coli strain M15/rep4 (Qiagen, Inc.) which contains multiple copies of the plasmid pREP4, which expresses  
20 the lacI repressor and also confers kanamycin resistance (Kan<sup>r</sup>). Transformants are identified by their ability to grow on LB plates and ampicillin/kanamycin resistant colonies are selected. Plasmid DNA is isolated and confirmed by restriction analysis.

Clones containing the desired constructs are grown overnight (O/N) in liquid culture in LB media supplemented with both Amp (100 ug/ml) and Kan (25 ug/ml).  
25 The O/N culture is used to inoculate a large culture at a ratio of 1:100 to 1:250. The cells are grown to an optical density 600 (O.D.<sup>600</sup>) of between 0.4 and 0.6. IPTG (Isopropyl-B-D-thiogalacto pyranoside) is then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression.

30 Cells are grown for an extra 3 to 4 hours. Cells are then harvested by centrifugation (20 mins at 6000Xg). The cell pellet is solubilized in the chaotropic agent 6 Molar Guanidine HCl by stirring for 3-4 hours at 4°C. The cell debris is removed by centrifugation, and the supernatant containing the polypeptide is loaded onto a nickel-nitrilo-tri-acetic acid ("Ni-NTA") affinity resin column (available from  
35 QIAGEN, Inc., *supra*). Proteins with a 6 x His tag bind to the Ni-NTA resin with high

affinity and can be purified in a simple one-step procedure (for details see: The QIAexpressionist (1995) QIAGEN, Inc., *supra*).

5 Briefly, the supernatant is loaded onto the column in 6 M guanidine-HCl, pH 8, the column is first washed with 10 volumes of 6 M guanidine-HCl, pH 8, then washed with 10 volumes of 6 M guanidine-HCl pH 6, and finally the polypeptide is eluted with 6 M guanidine-HCl, pH 5.

The purified protein is then renatured by dialyzing it against phosphate-buffered saline (PBS) or 50 mM Na-acetate, pH 6 buffer plus 200 mM NaCl. Alternatively, the protein can be successfully refolded while immobilized on the Ni-NTA column. The  
10 recommended conditions are as follows: renature using a linear 6M-1M urea gradient in 500 mM NaCl, 20% glycerol, 20 mM Tris/HCl pH 7.4, containing protease inhibitors. The renaturation should be performed over a period of 1.5 hours or more. After renaturation the proteins are eluted by the addition of 250 mM imidazole. Imidazole is removed by a final dialyzing step against PBS or 50 mM sodium acetate pH 6 buffer  
15 plus 200 mM NaCl. The purified protein is stored at 4°C or frozen at -80°C.

In addition to the above expression vector, the present invention further includes an expression vector comprising phage operator and promoter elements operatively linked to a polynucleotide of the present invention, called pHE4a. (ATCC Accession  
20 Number 209645, deposited on February 25, 1998.) This vector contains: 1) a neomycinphosphotransferase gene as a selection marker, 2) an E. coli origin of replication, 3) a T5 phage promoter sequence, 4) two lac operator sequences, 5) a Shine-Delgarno sequence, and 6) the lactose operon repressor gene (*lacIq*). The origin of replication (*oriC*) is derived from pUC19 (LTI, Gaithersburg, MD). The promoter sequence and operator sequences are made synthetically.

25 DNA can be inserted into the pHEa by restricting the vector with NdeI and XbaI, BamHI, XhoI, or Asp718, running the restricted product on a gel, and isolating the larger fragment (the stuffer fragment should be about 310 base pairs). The DNA insert is generated according to the PCR protocol described in Example 1, using PCR primers having restriction sites for NdeI (5' primer) and XbaI, BamHI, XhoI, or  
30 Asp718 (3' primer). The PCR insert is gel purified and restricted with compatible enzymes. The insert and vector are ligated according to standard protocols.

The engineered vector could easily be substituted in the above protocol to express protein in a bacterial system.

**Example 6: Purification of a Polypeptide from an Inclusion Body**

The following alternative method can be used to purify a polypeptide expressed in *E. coli* when it is present in the form of inclusion bodies. Unless otherwise specified, all of the following steps are conducted at 4-10°C.

5        Upon completion of the production phase of the *E. coli* fermentation, the cell culture is cooled to 4-10°C and the cells harvested by continuous centrifugation at 15,000 rpm (Heraeus Sepatech). On the basis of the expected yield of protein per unit weight of cell paste and the amount of purified protein required, an appropriate amount of cell paste, by weight, is suspended in a buffer solution containing 100 mM Tris, 50  
10    mM EDTA, pH 7.4. The cells are dispersed to a homogeneous suspension using a high shear mixer.

      The cells are then lysed by passing the solution through a microfluidizer (Microfluidics, Corp. or APV Gaulin, Inc.) twice at 4000-6000 psi. The homogenate is then mixed with NaCl solution to a final concentration of 0.5 M NaCl, followed by  
15    centrifugation at 7000 xg for 15 min. The resultant pellet is washed again using 0.5M NaCl, 100 mM Tris, 50 mM EDTA, pH 7.4.

      The resulting washed inclusion bodies are solubilized with 1.5 M guanidine hydrochloride (GuHCl) for 2-4 hours. After 7000 xg centrifugation for 15 min., the pellet is discarded and the polypeptide containing supernatant is incubated at 4°C  
20    overnight to allow further GuHCl extraction.

      Following high speed centrifugation (30,000 xg) to remove insoluble particles, the GuHCl solubilized protein is refolded by quickly mixing the GuHCl extract with 20 volumes of buffer containing 50 mM sodium, pH 4.5, 150 mM NaCl, 2 mM EDTA by vigorous stirring. The refolded diluted protein solution is kept at 4°C without mixing  
25    for 12 hours prior to further purification steps.

      To clarify the refolded polypeptide solution, a previously prepared tangential filtration unit equipped with 0.16 µm membrane filter with appropriate surface area (e.g., Filtron), equilibrated with 40 mM sodium acetate, pH 6.0 is employed. The filtered sample is loaded onto a cation exchange resin (e.g., Poros HS-50, Perseptive  
30    Biosystems). The column is washed with 40 mM sodium acetate, pH 6.0 and eluted with 250 mM, 500 mM, 1000 mM, and 1500 mM NaCl in the same buffer, in a stepwise manner. The absorbance at 280 nm of the effluent is continuously monitored. Fractions are collected and further analyzed by SDS-PAGE.

Fractions containing the polypeptide are then pooled and mixed with 4 volumes of water. The diluted sample is then loaded onto a previously prepared set of tandem columns of strong anion (Poros HQ-50, Perseptive Biosystems) and weak anion (Poros CM-20, Perseptive Biosystems) exchange resins. The columns are equilibrated with 40 mM sodium acetate, pH 6.0. Both columns are washed with 40 mM sodium acetate, pH 6.0, 200 mM NaCl. The CM-20 column is then eluted using a 10 column volume linear gradient ranging from 0.2 M NaCl, 50 mM sodium acetate, pH 6.0 to 1.0 M NaCl, 50 mM sodium acetate, pH 6.5. Fractions are collected under constant  $A_{280}$  monitoring of the effluent. Fractions containing the polypeptide (determined, for instance, by 16% SDS-PAGE) are then pooled.

The resultant polypeptide should exhibit greater than 95% purity after the above refolding and purification steps. No major contaminant bands should be observed from Commassie blue stained 16% SDS-PAGE gel when 5  $\mu$ g of purified protein is loaded. The purified protein can also be tested for endotoxin/LPS contamination, and typically the LPS content is less than 0.1 ng/ml according to LAL assays.

#### **Example 7: Cloning and Expression of a Polypeptide in a Baculovirus Expression System**

In this example, the plasmid shuttle vector pA2 is used to insert a polynucleotide into a baculovirus to express a polypeptide. This expression vector contains the strong polyhedrin promoter of the *Autographa californica* nuclear polyhedrosis virus (AcMNPV) followed by convenient restriction sites such as BamHI, Xba I and Asp718. The polyadenylation site of the simian virus 40 ("SV40") is used for efficient polyadenylation. For easy selection of recombinant virus, the plasmid contains the beta-galactosidase gene from *E. coli* under control of a weak *Drosophila* promoter in the same orientation, followed by the polyadenylation signal of the polyhedrin gene. The inserted genes are flanked on both sides by viral sequences for cell-mediated homologous recombination with wild-type viral DNA to generate a viable virus that express the cloned polynucleotide.

Many other baculovirus vectors can be used in place of the vector above, such as pAc373, pVL941, and pAcIM1, as one skilled in the art would readily appreciate, as long as the construct provides appropriately located signals for transcription, translation, secretion and the like, including a signal peptide and an in-frame AUG as required. Such vectors are described, for instance, in Luckow et al., *Virology* 170:31-39 (1989).

Specifically, the cDNA sequence contained in the deposited clone, including the AUG initiation codon and the naturally associated leader sequence identified in Table 1, is amplified using the PCR protocol described in Example 1. If the naturally occurring signal sequence is used to produce the secreted protein, the pA2 vector does not need a second signal peptide. Alternatively, the vector can be modified (pA2 GP) to include a baculovirus leader sequence, using the standard methods described in Summers et al., "A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures," Texas Agricultural Experimental Station Bulletin No. 1555 (1987).

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("GeneClean," BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with appropriate restriction enzymes and again purified on a 1% agarose gel.

The plasmid is digested with the corresponding restriction enzymes and optionally, can be dephosphorylated using calf intestinal phosphatase, using routine procedures known in the art. The DNA is then isolated from a 1% agarose gel using a commercially available kit ("GeneClean" BIO 101 Inc., La Jolla, Ca.).

The fragment and the dephosphorylated plasmid are ligated together with T4 DNA ligase. *E. coli* HB101 or other suitable *E. coli* hosts such as XL-1 Blue (Stratagene Cloning Systems, La Jolla, CA) cells are transformed with the ligation mixture and spread on culture plates. Bacteria containing the plasmid are identified by digesting DNA from individual colonies and analyzing the digestion product by gel electrophoresis. The sequence of the cloned fragment is confirmed by DNA sequencing.

Five  $\mu$ g of a plasmid containing the polynucleotide is co-transfected with 1.0  $\mu$ g of a commercially available linearized baculovirus DNA ("BaculoGold™ baculovirus DNA", Pharmingen, San Diego, CA), using the lipofection method described by Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413-7417 (1987). One  $\mu$ g of BaculoGold™ virus DNA and 5  $\mu$ g of the plasmid are mixed in a sterile well of a microtiter plate containing 50  $\mu$ l of serum-free Grace's medium (Life Technologies Inc., Gaithersburg, MD). Afterwards, 10  $\mu$ l Lipofectin plus 90  $\mu$ l Grace's medium are added, mixed and incubated for 15 minutes at room temperature. Then the transfection mixture is added drop-wise to Sf9 insect cells (ATCC CRL 1711) seeded in a 35 mm tissue culture plate with 1 ml Grace's medium without serum. The plate is then incubated for 5 hours at 27° C. The transfection solution is then removed from the plate and 1 ml of Grace's insect medium supplemented with 10% fetal calf serum is added. Cultivation is then continued at 27° C for four days.

After four days the supernatant is collected and a plaque assay is performed, as described by Summers and Smith, *supra*. An agarose gel with "Blue Gal" (Life



Technologies Inc., Gaithersburg) is used to allow easy identification and isolation of gal-expressing clones, which produce blue-stained plaques. (A detailed description of a "plaque assay" of this type can also be found in the user's guide for insect cell culture and baculovirology distributed by Life Technologies Inc., Gaithersburg, page 9-10.)

- 5 After appropriate incubation, blue stained plaques are picked with the tip of a micropipettor (e.g., Eppendorf). The agar containing the recombinant viruses is then resuspended in a microcentrifuge tube containing 200  $\mu$ l of Grace's medium and the suspension containing the recombinant baculovirus is used to infect Sf9 cells seeded in 35 mm dishes. Four days later the supernatants of these culture dishes are harvested  
10 and then they are stored at 4° C.

- To verify the expression of the polypeptide, Sf9 cells are grown in Grace's medium supplemented with 10% heat-inactivated FBS. The cells are infected with the recombinant baculovirus containing the polynucleotide at a multiplicity of infection ("MOI") of about 2. If radiolabeled proteins are desired, 6 hours later the medium is  
15 removed and is replaced with SF900 II medium minus methionine and cysteine (available from Life Technologies Inc., Rockville, MD). After 42 hours, 5  $\mu$ Ci of  $^{35}$ S-methionine and 5  $\mu$ Ci  $^{35}$ S-cysteine (available from Amersham) are added. The cells are further incubated for 16 hours and then are harvested by centrifugation. The proteins in the supernatant as well as the intracellular proteins are analyzed by SDS-PAGE  
20 followed by autoradiography (if radiolabeled).

Microsequencing of the amino acid sequence of the amino terminus of purified protein may be used to determine the amino terminal sequence of the produced protein.

#### **Example 8: Expression of a Polypeptide in Mammalian Cells**

- The polypeptide of the present invention can be expressed in a mammalian cell.  
25 A typical mammalian expression vector contains a promoter element, which mediates the initiation of transcription of mRNA, a protein coding sequence, and signals required for the termination of transcription and polyadenylation of the transcript. Additional elements include enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription is achieved  
30 with the early and late promoters from SV40, the long terminal repeats (LTRs) from Retroviruses, e.g., RSV, HTLV, HIV and the early promoter of the cytomegalovirus (CMV). However, cellular elements can also be used (e.g., the human actin promoter).

- Suitable expression vectors for use in practicing the present invention include, for example, vectors such as pSVL and pMSG (Pharmacia, Uppsala, Sweden),  
35 pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146), pBC12MI (ATCC 67109), pCMVSPORT 2.0, and pCMVSPORT 3.0. Mammalian host cells that could be used

include, human Hela, 293, H9 and Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV1, quail QC1-3 cells, mouse L cells and Chinese hamster ovary (CHO) cells.

Alternatively, the polypeptide can be expressed in stable cell lines containing the polynucleotide integrated into a chromosome. The co-transfection with a selectable marker such as dhfr, gpt, neomycin, hygromycin allows the identification and isolation of the transfected cells.

The transfected gene can also be amplified to express large amounts of the encoded protein. The DHFR (dihydrofolate reductase) marker is useful in developing cell lines that carry several hundred or even several thousand copies of the gene of interest. (See, e.g., Alt, F. W., et al., *J. Biol. Chem.* 253:1357-1370 (1978); Hamlin, J. L. and Ma, C., *Biochem. et Biophys. Acta*, 1097:107-143 (1990); Page, M. J. and Sydenham, M. A., *Biotechnology* 9:64-68 (1991).) Another useful selection marker is the enzyme glutamine synthase (GS) (Murphy et al., *Biochem J.* 227:277-279 (1991); Bebbington et al., *Bio/Technology* 10:169-175 (1992). Using these markers, the mammalian cells are grown in selective medium and the cells with the highest resistance are selected. These cell lines contain the amplified gene(s) integrated into a chromosome. Chinese hamster ovary (CHO) and NSO cells are often used for the production of proteins.

Derivatives of the plasmid pSV2-dhfr (ATCC Accession No. 37146), the expression vectors pC4 (ATCC Accession No. 209646) and pC6 (ATCC Accession No. 209647) contain the strong promoter (LTR) of the Rous Sarcoma Virus (Cullen et al., *Molecular and Cellular Biology*, 438-447 (March, 1985)) plus a fragment of the CMV-enhancer (Boshart et al., *Cell* 41:521-530 (1985).) Multiple cloning sites, e.g., with the restriction enzyme cleavage sites BamHI, XbaI and Asp718, facilitate the cloning of the gene of interest. The vectors also contain the 3' intron, the polyadenylation and termination signal of the rat preproinsulin gene, and the mouse DHFR gene under control of the SV40 early promoter.

Specifically, the plasmid pC6, for example, is digested with appropriate restriction enzymes and then dephosphorylated using calf intestinal phosphates by procedures known in the art. The vector is then isolated from a 1% agarose gel.

A polynucleotide of the present invention is amplified according to the protocol outlined in Example 1. If the naturally occurring signal sequence is used to produce the secreted protein, the vector does not need a second signal peptide. Alternatively, if the naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence. (See, e.g., WO 96/34891.)

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("Geneclean," BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with appropriate restriction enzymes and again purified on a 1% agarose gel.

5 The amplified fragment is then digested with the same restriction enzyme and purified on a 1% agarose gel. The isolated fragment and the dephosphorylated vector are then ligated with T4 DNA ligase. *E. coli* HB101 or XL-1 Blue cells are then transformed and bacteria are identified that contain the fragment inserted into plasmid pC6 using, for instance, restriction enzyme analysis.

10 Chinese hamster ovary cells lacking an active DHFR gene is used for transfection. Five  $\mu$ g of the expression plasmid pC6 is cotransfected with 0.5  $\mu$ g of the plasmid pSVneo using lipofectin (Felgner et al., *supra*). The plasmid pSV2-neo contains a dominant selectable marker, the *neo* gene from Tn5 encoding an enzyme that confers resistance to a group of antibiotics including G418. The cells are seeded in alpha minus MEM supplemented with 1 mg/ml G418. After 2 days, the cells are  
15 trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) in alpha minus MEM supplemented with 10, 25, or 50 ng/ml of methotrexate plus 1 mg/ml G418. After about 10-14 days single clones are trypsinized and then seeded in 6-well petri dishes or 10 ml flasks using different concentrations of methotrexate (50 nM, 100 nM, 200 nM, 400 nM, 800 nM). Clones growing at the highest concentrations of  
20 methotrexate are then transferred to new 6-well plates containing even higher concentrations of methotrexate (1  $\mu$ M, 2  $\mu$ M, 5  $\mu$ M, 10 mM, 20 mM). The same procedure is repeated until clones are obtained which grow at a concentration of 100 - 200  $\mu$ M. Expression of the desired gene product is analyzed, for instance, by SDS-PAGE and Western blot or by reversed phase HPLC analysis.

25

### Example 9: Protein Fusions

The polypeptides of the present invention are preferably fused to other proteins. These fusion proteins can be used for a variety of applications. For example, fusion of the present polypeptides to His-tag, HA-tag, protein A, IgG domains, and maltose  
30 binding protein facilitates purification. (See Example 5; see also EP A 394,827; Traunecker, et al., Nature 331:84-86 (1988).) Similarly, fusion to IgG-1, IgG-3, and albumin increases the half-life time in vivo. Nuclear localization signals fused to the polypeptides of the present invention can target the protein to a specific subcellular localization, while covalent heterodimer or homodimers can increase or decrease the  
35 activity of a fusion protein. Fusion proteins can also create chimeric molecules having more than one function. Finally, fusion proteins can increase solubility and/or stability of the fused protein compared to the non-fused protein. All of the types of fusion

proteins described above can be made by modifying the following protocol, which outlines the fusion of a polypeptide to an IgG molecule, or the protocol described in Example 5.

5 Briefly, the human Fc portion of the IgG molecule can be PCR amplified, using primers that span the 5' and 3' ends of the sequence described below. These primers also should have convenient restriction enzyme sites that will facilitate cloning into an expression vector, preferably a mammalian expression vector.

10 For example, if pC4 (Accession No. 209646) is used, the human Fc portion can be ligated into the BamHI cloning site. Note that the 3' BamHI site should be destroyed. Next, the vector containing the human Fc portion is re-restricted with BamHI, linearizing the vector, and a polynucleotide of the present invention, isolated by the PCR protocol described in Example 1, is ligated into this BamHI site. Note that the polynucleotide is cloned without a stop codon, otherwise a fusion protein will not be produced.

15 If the naturally occurring signal sequence is used to produce the secreted protein, pC4 does not need a second signal peptide. Alternatively, if the naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence. (See, e.g., WO 96/34891.)

20 Human IgG Fc region:

GGGATCCGGAGCCCAAATCTTCTGACAAAACTCACACATGCCCACCGTGCC  
CAGCACCTGAATTCGAGGGTGCACCGTCAGTCTTCCTCTTCCCCCAAAC  
CAAGGACACCCTCATGATCTCCCGGACTCCTGAGGTCACATGCGTGGTGGT  
GGACGTAAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACG  
25 GCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAAC  
AGCACGTACCGTGTGGTACGCGTCCTCACCGTCCTGCACCAGGACTGGCTG  
AATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAACCCCC  
ATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGT  
GTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCT  
30 GACCTGCCTGGTCAAAGGCTTCTATCCAAGCGACATCGCCGTGGAGTGGGA  
GAGCAATGGGCAGCCGGAGAACAACACTACAAGACCACGCCTCCCGTGCTGG  
ACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGGACAAGAGCA  
GGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGC  
ACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGAGTGC  
35 GACGGCCGCGACTCTAGAGGAT (SEQ ID NO:1)

**Example 10: Production of an Antibody from a Polypeptide**

The antibodies of the present invention can be prepared by a variety of methods. (See, Current Protocols, Chapter 2.) For example, cells expressing a polypeptide of the present invention is administered to an animal to induce the production of sera containing polyclonal antibodies. In a preferred method, a preparation of the secreted protein is prepared and purified to render it substantially free of natural contaminants. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity.

In the most preferred method, the antibodies of the present invention are monoclonal antibodies (or protein binding fragments thereof). Such monoclonal antibodies can be prepared using hybridoma technology. (Köhler et al., Nature 256:495 (1975); Köhler et al., Eur. J. Immunol. 6:511 (1976); Köhler et al., Eur. J. Immunol. 6:292 (1976); Hammerling et al., in: Monoclonal Antibodies and T-Cell Hybridomas, Elsevier, N.Y., pp. 563-681 (1981).) In general, such procedures involve immunizing an animal (preferably a mouse) with polypeptide or, more preferably, with a secreted polypeptide-expressing cell. Such cells may be cultured in any suitable tissue culture medium; however, it is preferable to culture cells in Earle's modified Eagle's medium supplemented with 10% fetal bovine serum (inactivated at about 56°C), and supplemented with about 10 g/l of nonessential amino acids, about 1,000 U/ml of penicillin, and about 100 µg/ml of streptomycin.

The splenocytes of such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (SP2O), available from the ATCC. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands et al. (Gastroenterology 80:225-232 (1981).) The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the polypeptide.

Alternatively, additional antibodies capable of binding to the polypeptide can be produced in a two-step procedure using anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and therefore, it is possible to obtain an antibody which binds to a second antibody. In accordance with this method, protein specific antibodies are used to immunize an animal, preferably a mouse. The splenocytes of such an animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones which produce an antibody whose ability to bind to the protein-specific antibody can be blocked by the polypeptide.

Such antibodies comprise anti-idiotypic antibodies to the protein-specific antibody and can be used to immunize an animal to induce formation of further protein-specific antibodies.

5 It will be appreciated that Fab and F(ab')<sub>2</sub> and other fragments of the antibodies of the present invention may be used according to the methods disclosed herein. Such fragments are typically produced by proteolytic cleavage, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')<sub>2</sub> fragments). Alternatively, secreted protein-binding fragments can be produced through the application of recombinant DNA technology or through synthetic chemistry.

10 For in vivo use of antibodies in humans, it may be preferable to use "humanized" chimeric monoclonal antibodies. Such antibodies can be produced using genetic constructs derived from hybridoma cells producing the monoclonal antibodies described above. Methods for producing chimeric antibodies are known in the art. (See, for review, Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Cabilly et al., U.S. Patent No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494; Neuberger et al., WO 8601533; Robinson et al., WO 8702671; Boulianne et al., Nature 312:643 (1984); Neuberger et al., Nature 314:268 (1985).)

20 **Example 11: Production Of Secreted Protein For High-Throughput Screening Assays**

The following protocol produces a supernatant containing a polypeptide to be tested. This supernatant can then be used in the Screening Assays described in Examples 13-20.

25 First, dilute Poly-D-Lysine (644 587 Boehringer-Mannheim) stock solution (1mg/ml in PBS) 1:20 in PBS (w/o calcium or magnesium 17-516F Biowhittaker) for a working solution of 50ug/ml. Add 200 ul of this solution to each well (24 well plates) and incubate at RT for 20 minutes. Be sure to distribute the solution over each well (note: a 12-channel pipetter may be used with tips on every other channel). Aspirate off the Poly-D-Lysine solution and rinse with 1ml PBS (Phosphate Buffered Saline). The PBS should remain in the well until just prior to plating the cells and plates may be poly-lysine coated in advance for up to two weeks.

30 Plate 293T cells (do not carry cells past P+20) at  $2 \times 10^5$  cells/well in .5ml DMEM(Dulbecco's Modified Eagle Medium)(with 4.5 G/L glucose and L-glutamine (12-604F Biowhittaker))/10% heat inactivated FBS(14-503F Biowhittaker)/1x Penstrep(17-602E Biowhittaker). Let the cells grow overnight.

The next day, mix together in a sterile solution basin: 300 ul Lipofectamine (18324-012 Gibco/BRL) and 5ml Optimem I (31985070 Gibco/BRL)/96-well plate. With a small volume multi-channel pipetter, aliquot approximately 2ug of an expression vector containing a polynucleotide insert, produced by the methods described in Examples 8 or 9, into an appropriately labeled 96-well round bottom plate. With a multi-channel pipetter, add 50ul of the Lipofectamine/Optimem I mixture to each well. Pipette up and down gently to mix. Incubate at RT 15-45 minutes. After about 20 minutes, use a multi-channel pipetter to add 150ul Optimem I to each well. As a control, one plate of vector DNA lacking an insert should be transfected with each set of transfections.

Preferably, the transfection should be performed by tag-teaming the following tasks. By tag-teaming, hands on time is cut in half, and the cells do not spend too much time on PBS. First, person A aspirates off the media from four 24-well plates of cells, and then person B rinses each well with .5-1ml PBS. Person A then aspirates off PBS rinse, and person B, using a 12-channel pipetter with tips on every other channel, adds the 200ul of DNA/Lipofectamine/Optimem I complex to the odd wells first, then to the even wells, to each row on the 24-well plates. Incubate at 37°C for 6 hours.

While cells are incubating, prepare appropriate media, either 1%BSA in DMEM with 1x penstrep, or CHO-5 media (116.6 mg/L of CaCl<sub>2</sub> (anhyd); 0.00130 mg/L CuSO<sub>4</sub>·5H<sub>2</sub>O; 0.050 mg/L of Fe(NO<sub>3</sub>)<sub>3</sub>·9H<sub>2</sub>O; 0.417 mg/L of FeSO<sub>4</sub>·7H<sub>2</sub>O; 311.80 mg/L of KCl; 28.64 mg/L of MgCl<sub>2</sub>; 48.84 mg/L of MgSO<sub>4</sub>; 6995.50 mg/L of NaCl; 2400.0 mg/L of NaHCO<sub>3</sub>; 62.50 mg/L of NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O; 71.02 mg/L of Na<sub>2</sub>HPO<sub>4</sub>; .4320 mg/L of ZnSO<sub>4</sub>·7H<sub>2</sub>O; .002 mg/L of Arachidonic Acid; 1.022 mg/L of Cholesterol; .070 mg/L of DL-alpha-Tocopherol-Acetate; 0.0520 mg/L of Linoleic Acid; 0.010 mg/L of Linolenic Acid; 0.010 mg/L of Myristic Acid; 0.010 mg/L of Oleic Acid; 0.010 mg/L of Palmitic Acid; 0.010 mg/L of Palmitic Acid; 100 mg/L of Pluronic F-68; 0.010 mg/L of Stearic Acid; 2.20 mg/L of Tween 80; 4551 mg/L of D-Glucose; 130.85 mg/ml of L- Alanine; 147.50 mg/ml of L-Arginine-HCL; 7.50 mg/ml of L-Asparagine-H<sub>2</sub>O; 6.65 mg/ml of L-Aspartic Acid; 29.56 mg/ml of L-Cystine-2HCL-H<sub>2</sub>O; 31.29 mg/ml of L-Cystine-2HCL; 7.35 mg/ml of L-Glutamic Acid; 365.0 mg/ml of L-Glutamine; 18.75 mg/ml of Glycine; 52.48 mg/ml of L-Histidine-HCL-H<sub>2</sub>O; 106.97 mg/ml of L-Isoleucine; 111.45 mg/ml of L-Leucine; 163.75 mg/ml of L-Lysine HCL; 32.34 mg/ml of L-Methionine; 68.48 mg/ml of L-Phenylalanine; 40.0 mg/ml of L-Proline; 26.25 mg/ml of L-Serine; 101.05 mg/ml of L-Threonine; 19.22 mg/ml of L-Tryptophan; 91.79 mg/ml of L-Tyrosine-2Na-2H<sub>2</sub>O; 99.65 mg/ml of L-Valine; 0.0035 mg/L of Biotin; 3.24 mg/L of D-Ca Pantothenate; 11.78 mg/L of

Choline Chloride; 4.65 mg/L of Folic Acid; 15.60 mg/L of i-Inositol; 3.02 mg/L of Niacinamide; 3.00 mg/L of Pyridoxal HCL; 0.031 mg/L of Pyridoxine HCL; 0.319 mg/L of Riboflavin; 3.17 mg/L of Thiamine HCL; 0.365 mg/L of Thymidine; and 0.680 mg/L of Vitamin B<sub>12</sub>; 25 mM of HEPES Buffer; 2.39 mg/L of Na Hypoxanthine; 5 0.105 mg/L of Lipoic Acid; 0.081 mg/L of Sodium Putrescine-2HCL; 55.0 mg/L of Sodium Pyruvate; 0.0067 mg/L of Sodium Selenite; 20uM of Ethanolamine; 0.122 mg/L of Ferric Citrate; 41.70 mg/L of Methyl-B-Cyclodextrin complexed with Linoleic Acid; 33.33 mg/L of Methyl-B-Cyclodextrin complexed with Oleic Acid; and 10 mg/L of Methyl-B-Cyclodextrin complexed with Retinal) with 2mm glutamine and 1x penstrep. (BSA (81-068-3 Bayer) 100gm dissolved in 1L DMEM for a 10% BSA stock solution). Filter the media and collect 50 ul for endotoxin assay in 15ml polystyrene conical.

The transfection reaction is terminated, preferably by tag-teaming, at the end of the incubation period. Person A aspirates off the transfection media, while person B 15 adds 1.5ml appropriate media to each well. Incubate at 37°C for 45 or 72 hours depending on the media used: 1%BSA for 45 hours or CHO-5 for 72 hours.

On day four, using a 300ul multichannel pipetter, aliquot 600ul in one 1ml deep well plate and the remaining supernatant into a 2ml deep well. The supernatants from each well can then be used in the assays described in Examples 13-20.

20 It is specifically understood that when activity is obtained in any of the assays described below using a supernatant, the activity originates from either the polypeptide directly (e.g., as a secreted protein) or by the polypeptide inducing expression of other proteins, which are then secreted into the supernatant. Thus, the invention further provides a method of identifying the protein in the supernatant characterized by an 25 activity in a particular assay.

#### **Example 12: Construction of GAS Reporter Construct**

One signal transduction pathway involved in the differentiation and proliferation of cells is called the Jaks-STATs pathway. Activated proteins in the Jaks-STATs 30 pathway bind to gamma activation site "GAS" elements or interferon-sensitive responsive element ("ISRE"), located in the promoter of many genes. The binding of a protein to these elements alter the expression of the associated gene.

GAS and ISRE elements are recognized by a class of transcription factors called Signal Transducers and Activators of Transcription, or "STATs." There are six 35 members of the STATs family. Stat1 and Stat3 are present in many cell types, as is Stat2 (as response to IFN-alpha is widespread). Stat4 is more restricted and is not in



many cell types though it has been found in T helper class 1, cells after treatment with IL-12. Stat5 was originally called mammary growth factor, but has been found at higher concentrations in other cells including myeloid cells. It can be activated in tissue culture cells by many cytokines.

5       The STATs are activated to translocate from the cytoplasm to the nucleus upon tyrosine phosphorylation by a set of kinases known as the Janus Kinase ("Jaks") family. Jaks represent a distinct family of soluble tyrosine kinases and include Tyk2, Jak1, Jak2, and Jak3. These kinases display significant sequence similarity and are generally catalytically inactive in resting cells.

10       The Jaks are activated by a wide range of receptors summarized in the Table below. (Adapted from review by Schidler and Darnell, Ann. Rev. Biochem. 64:621-51 (1995).) A cytokine receptor family, capable of activating Jaks, is divided into two groups: (a) Class 1 includes receptors for IL-2, IL-3, IL-4, IL-6, IL-7, IL-9, IL-11, IL-12, IL-15, Epo, PRL, GH, G-CSF, GM-CSF, LIF, CNTF, and thrombopoietin; and  
15       (b) Class 2 includes IFN-a, IFN-g, and IL-10. The Class 1 receptors share a conserved cysteine motif (a set of four conserved cysteines and one tryptophan) and a WSXWS motif (a membrane proximal region encoding Trp-Ser-Xxx-Trp-Ser (SEQ ID NO:2)).

20       Thus, on binding of a ligand to a receptor, Jaks are activated, which in turn activate STATs, which then translocate and bind to GAS elements. This entire process is encompassed in the Jaks-STATs signal transduction pathway.

25       Therefore, activation of the Jaks-STATs pathway, reflected by the binding of the GAS or the ISRE element, can be used to indicate proteins involved in the proliferation and differentiation of cells. For example, growth factors and cytokines are known to activate the Jaks-STATs pathway. (See Table below.) Thus, by using GAS elements linked to reporter molecules, activators of the Jaks-STATs pathway can be identified.

	<u>Ligand</u>	<u>tyk2</u>	<u>JAKs</u> <u>Jak1</u>	<u>Jak2</u>	<u>Jak3</u>	<u>STATS</u>	<u>GAS(elements) or ISRE</u>
5	<u>IFN family</u>						
	IFN-a/B	+	+	-	-	1,2,3	ISRE
	IFN-g		+	+	-	1	GAS (IRF1>Lys6>IFP)
	Il-10	+	?	?	-	1,3	
	<u>gp130 family</u>						
10	IL-6 (Pleiotrohic)	+	+	+	?	1,3	GAS (IRF1>Lys6>IFP)
	Il-11(Pleiotrohic)	?	+	?	?	1,3	
	OnM(Pleiotrohic)	?	+	+	?	1,3	
	LIF(Pleiotrohic)	?	+	+	?	1,3	
	CNTF(Pleiotrohic)	-/+	+	+	?	1,3	
15	G-CSF(Pleiotrohic)	?	+	?	?	1,3	
	IL-12(Pleiotrohic)	+	-	+	+	1,3	
	<u>g-C family</u>						
20	IL-2 (lymphocytes)	-	+	-	+	1,3,5	GAS
	IL-4 (lymph/myeloid)	-	+	-	+	6	GAS (IRF1 = IFP >>Ly6)(IgH)
	IL-7 (lymphocytes)	-	+	-	+	5	GAS
	IL-9 (lymphocytes)	-	+	-	+	5	GAS
	IL-13 (lymphocyte)	-	+	?	?	6	GAS
	IL-15	?	+	?	+	5	GAS
25	<u>gp140 family</u>						
	IL-3 (myeloid)	-	-	+	-	5	GAS (IRF1>IFP>>Ly6)
	IL-5 (myeloid)	-	-	+	-	5	GAS
	GM-CSF (myeloid)	-	-	+	-	5	GAS
30	<u>Growth hormone family</u>						
	GH	?	-	+	-	5	
	PRL	?	+/-	+	-	1,3,5	
	EPO	?	-	+	-	5	GAS(B-CAS>IRF1=IFP>>Ly6)
35	<u>Receptor Tyrosine Kinases</u>						
	EGF	?	+	+	-	1,3	GAS (IRF1)
	PDGF	?	+	+	-	1,3	
	CSF-1	?	+	+	-	1,3	GAS (not IRF1)
40							

To construct a synthetic GAS containing promoter element, which is used in the Biological Assays described in Examples 13-14, a PCR based strategy is employed to generate a GAS-SV40 promoter sequence. The 5' primer contains four tandem copies of the GAS binding site found in the IRF1 promoter and previously demonstrated to bind STATs upon induction with a range of cytokines (Rothman et al., Immunity 1:457-468 (1994).), although other GAS or ISRE elements can be used instead. The 5' primer also contains 18bp of sequence complementary to the SV40 early promoter sequence and is flanked with an XhoI site. The sequence of the 5' primer is:

5':GCGCCTCGAGATTTCCCCGAAATCTAGATTTCCCCGAAATGATTTCCCCG  
 10 AAATGATTTCCCCGAAATATCTGCCATCTCAATTAG:3' (SEQ ID NO:3)

The downstream primer is complementary to the SV40 promoter and is flanked with a Hind III site: 5':GCGGCAAGCTTTTGTCAAAGCCTAGGC:3' (SEQ ID NO:4)

PCR amplification is performed using the SV40 promoter template present in the B-gal:promoter plasmid obtained from Clontech. The resulting PCR fragment is digested with XhoI/Hind III and subcloned into BLSK2-. (Stratagene.) Sequencing with forward and reverse primers confirms that the insert contains the following sequence:5':CTCGAGATTTCCCCGAAATCTAGATTTCCCCGAAATGATTTCCCC  
 GAAATGATTTCCCCGAAATATCTGCCATCTCAATTAGTCAGCAACCATAGTC  
 20 CCGCCCCCTAACTCCGCCCATCCCGCCCCCTAACTCCGCCCAGTTCCGCCCATT  
 CTCGCCCCATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGC  
 CTCGGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTTGGAGGCCT  
 AGGCTTTTGCAAAAAGCTT:3' (SEQ ID NO:5)

With this GAS promoter element linked to the SV40 promoter, a GAS:SEAP2 reporter construct is next engineered. Here, the reporter molecule is a secreted alkaline phosphatase, or "SEAP." Clearly, however, any reporter molecule can be instead of SEAP, in this or in any of the other Examples. Well known reporter molecules that can be used instead of SEAP include chloramphenicol acetyltransferase (CAT), luciferase, alkaline phosphatase, B-galactosidase, green fluorescent protein (GFP), or any protein detectable by an antibody.

The above sequence confirmed synthetic GAS-SV40 promoter element is subcloned into the pSEAP-Promoter vector obtained from Clontech using HindIII and XhoI, effectively replacing the SV40 promoter with the amplified GAS:SV40 promoter element, to create the GAS-SEAP vector. However, this vector does not contain a neomycin resistance gene, and therefore, is not preferred for mammalian expression systems.

Thus, in order to generate mammalian stable cell lines expressing the GAS-SEAP reporter, the GAS-SEAP cassette is removed from the GAS-SEAP vector using Sall and NotI, and inserted into a backbone vector containing the neomycin resistance gene, such as pGFP-1 (Clontech), using these restriction sites in the multiple cloning site, to create the GAS-SEAP/Neo vector. Once this vector is transfected into mammalian cells, this vector can then be used as a reporter molecule for GAS binding as described in Examples 13-14.

Other constructs can be made using the above description and replacing GAS with a different promoter sequence. For example, construction of reporter molecules containing NFK-B and EGR promoter sequences are described in Examples 15 and 16. However, many other promoters can be substituted using the protocols described in these Examples. For instance, SRE, IL-2, NFAT, or Osteocalcin promoters can be substituted, alone or in combination (e.g., GAS/NF-KB/EGR, GAS/NF-KB, IL-2/NFAT, or NF-KB/GAS). Similarly, other cell lines can be used to test reporter construct activity, such as HELA (epithelial), HUVEC (endothelial), Reh (B-cell), Saos-2 (osteoblast), HUVAC (aortic), or Cardiomyocyte.

**Example 13: High-Throughput Screening Assay for T-cell Activity.**

The following protocol is used to assess T-cell activity by identifying factors, such as growth factors and cytokines, that may proliferate or differentiate T-cells. T-cell activity is assessed using the GAS/SEAP/Neo construct produced in Example 12. Thus, factors that increase SEAP activity indicate the ability to activate the Jaks-STATS signal transduction pathway. The T-cell used in this assay is Jurkat T-cells (ATCC Accession No. TIB-152), although Molt-3 cells (ATCC Accession No. CRL-1552) and Molt-4 cells (ATCC Accession No. CRL-1582) cells can also be used.

Jurkat T-cells are lymphoblastic CD4+ Th1 helper cells. In order to generate stable cell lines, approximately 2 million Jurkat cells are transfected with the GAS-SEAP/neo vector using DMRIE-C (Life Technologies)(transfection procedure described below). The transfected cells are seeded to a density of approximately 20,000 cells per well and transfectants resistant to 1 mg/ml gentamicin selected. Resistant colonies are expanded and then tested for their response to increasing concentrations of interferon gamma. The dose response of a selected clone is demonstrated.

Specifically, the following protocol will yield sufficient cells for 75 wells containing 200 ul of cells. Thus, it is either scaled up, or performed in multiple to generate sufficient cells for multiple 96 well plates. Jurkat cells are maintained in RPMI + 10% serum with 1%Pen-Strep. Combine 2.5 mls of OPTI-MEM (Life Technologies)

with 10 ug of plasmid DNA in a T25 flask. Add 2.5 ml OPTI-MEM containing 50 ul of DMRIE-C and incubate at room temperature for 15-45 mins.

- During the incubation period, count cell concentration, spin down the required number of cells ( $10^7$  per transfection), and resuspend in OPTI-MEM to a final  
5 concentration of  $10^7$  cells/ml. Then add 1ml of  $1 \times 10^7$  cells in OPTI-MEM to T25 flask and incubate at 37°C for 6 hrs. After the incubation, add 10 ml of RPMI + 15% serum.

The Jurkat:GAS-SEAP stable reporter lines are maintained in RPMI + 10% serum, 1 mg/ml Genticin, and 1% Pen-Strep. These cells are treated with supernatants containing a polypeptide as produced by the protocol described in Example 11.

- 10 On the day of treatment with the supernatant, the cells should be washed and resuspended in fresh RPMI + 10% serum to a density of 500,000 cells per ml. The exact number of cells required will depend on the number of supernatants being screened. For one 96 well plate, approximately 10 million cells (for 10 plates, 100 million cells) are required.

- 15 Transfer the cells to a triangular reservoir boat, in order to dispense the cells into a 96 well dish, using a 12 channel pipette. Using a 12 channel pipette, transfer 200 ul of cells into each well (therefore adding 100,000 cells per well).

- After all the plates have been seeded, 50 ul of the supernatants are transferred directly from the 96 well plate containing the supernatants into each well using a 12  
20 channel pipette. In addition, a dose of exogenous interferon gamma (0.1, 1.0, 10 ng) is added to wells H9, H10, and H11 to serve as additional positive controls for the assay.

- The 96 well dishes containing Jurkat cells treated with supernatants are placed in an incubator for 48 hrs (note: this time is variable between 48-72 hrs). 35 ul samples  
25 from each well are then transferred to an opaque 96 well plate using a 12 channel pipette. The opaque plates should be covered (using sellophane covers) and stored at -20°C until SEAP assays are performed according to Example 17. The plates containing the remaining treated cells are placed at 4°C and serve as a source of material for repeating the assay on a specific well if desired.

- 30 As a positive control, 100 Unit/ml interferon gamma can be used which is known to activate Jurkat T cells. Over 30 fold induction is typically observed in the positive control wells.

**Example 14: High-Throughput Screening Assay Identifying Myeloid Activity**

The following protocol is used to assess myeloid activity by identifying factors, such as growth factors and cytokines, that may proliferate or differentiate myeloid cells.

- 5 Myeloid cell activity is assessed using the GAS/SEAP/Neo construct produced in Example 12. Thus, factors that increase SEAP activity indicate the ability to activate the Jaks-STATS signal transduction pathway. The myeloid cell used in this assay is U937, a pre-monocyte cell line, although TF-1, HL60, or KG1 can be used.

- 10 To transiently transfect U937 cells with the GAS/SEAP/Neo construct produced in Example 12, a DEAE-Dextran method (Kharbanda et. al., 1994, Cell Growth & Differentiation, 5:259-265) is used. First, harvest  $2 \times 10^7$  U937 cells and wash with PBS. The U937 cells are usually grown in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum (FBS) supplemented with 100 units/ml penicillin and 100 mg/ml streptomycin.

- 15 Next, suspend the cells in 1 ml of 20 mM Tris-HCl (pH 7.4) buffer containing 0.5 mg/ml DEAE-Dextran, 8 ug GAS-SEAP2 plasmid DNA, 140 mM NaCl, 5 mM KCl, 375 uM  $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ , 1 mM  $\text{MgCl}_2$ , and 675 uM  $\text{CaCl}_2$ . Incubate at  $37^\circ\text{C}$  for 45 min.

- 20 Wash the cells with RPMI 1640 medium containing 10% FBS and then resuspend in 10 ml complete medium and incubate at  $37^\circ\text{C}$  for 36 hr.

The GAS-SEAP/U937 stable cells are obtained by growing the cells in 400 ug/ml G418. The G418-free medium is used for routine growth but every one to two months, the cells should be re-grown in 400 ug/ml G418 for couple of passages.

- 25 These cells are tested by harvesting  $1 \times 10^8$  cells (this is enough for ten 96-well plates assay) and wash with PBS. Suspend the cells in 200 ml above described growth medium, with a final density of  $5 \times 10^5$  cells/ml. Plate 200 ul cells per well in the 96-well plate (or  $1 \times 10^5$  cells/well).

- 30 Add 50 ul of the supernatant prepared by the protocol described in Example 11. Incubate at  $37^\circ\text{C}$  for 48 to 72 hr. As a positive control, 100 Unit/ml interferon gamma can be used which is known to activate U937 cells. Over 30 fold induction is typically observed in the positive control wells. SEAP assay the supernatant according to the protocol described in Example 17.

**Example 15: High-Throughput Screening Assay Identifying Neuronal Activity.**

When cells undergo differentiation and proliferation, a group of genes are activated through many different signal transduction pathways. One of these genes, EGR1 (early growth response gene 1), is induced in various tissues and cell types upon activation. The promoter of EGR1 is responsible for such induction. Using the EGR1 promoter linked to reporter molecules, activation of cells can be assessed.

Particularly, the following protocol is used to assess neuronal activity in PC12 cell lines. PC12 cells (rat phenochromocytoma cells) are known to proliferate and/or differentiate by activation with a number of mitogens, such as TPA (tetradecanoyl phorbol acetate), NGF (nerve growth factor), and EGF (epidermal growth factor). The EGR1 gene expression is activated during this treatment. Thus, by stably transfecting PC12 cells with a construct containing an EGR promoter linked to SEAP reporter, activation of PC12 cells can be assessed.

The EGR/SEAP reporter construct can be assembled by the following protocol. The EGR-1 promoter sequence (-633 to +1)(Sakamoto K et al., Oncogene 6:867-871 (1991)) can be PCR amplified from human genomic DNA using the following primers:

5' GCGCTCGAGGGATGACAGCGATAGAACCCCGG -3' (SEQ ID NO:6)

5' GCGAAGCTTCGCGACTCCCGGATCCGCCTC-3' (SEQ ID NO:7)

Using the GAS:SEAP/Neo vector produced in Example 12, EGR1 amplified product can then be inserted into this vector. Linearize the GAS:SEAP/Neo vector using restriction enzymes XhoI/HindIII, removing the GAS/SV40 stuffer. Restrict the EGR1 amplified product with these same enzymes. Ligate the vector and the EGR1 promoter.

To prepare 96 well-plates for cell culture, two mls of a coating solution (1:30 dilution of collagen type I (Upstate Biotech Inc. Cat#08-115) in 30% ethanol (filter sterilized)) is added per one 10 cm plate or 50 ml per well of the 96-well plate, and allowed to air dry for 2 hr.

PC12 cells are routinely grown in RPMI-1640 medium (Bio Whittaker) containing 10% horse serum (JRH BIOSCIENCES, Cat. # 12449-78P), 5% heat-inactivated fetal bovine serum (FBS) supplemented with 100 units/ml penicillin and 100 ug/ml streptomycin on a precoated 10 cm tissue culture dish. One to four split is done every three to four days. Cells are removed from the plates by scraping and resuspended with pipetting up and down for more than 15 times.

Transfect the EGR/SEAP/Neo construct into PC12 using the Lipofectamine protocol described in Example 11. EGR-SEAP/PC12 stable cells are obtained by growing the cells in 300 ug/ml G418. The G418-free medium is used for routine

growth but every one to two months, the cells should be re-grown in 300 ug/ml G418 for couple of passages.

To assay for neuronal activity, a 10 cm plate with cells around 70 to 80% confluent is screened by removing the old medium. Wash the cells once with PBS (Phosphate buffered saline). Then starve the cells in low serum medium (RPMI-1640 containing 1% horse serum and 0.5% FBS with antibiotics) overnight.

The next morning, remove the medium and wash the cells with PBS. Scrape off the cells from the plate, suspend the cells well in 2 ml low serum medium. Count the cell number and add more low serum medium to reach final cell density as  $5 \times 10^5$  cells/ml.

Add 200 ul of the cell suspension to each well of 96-well plate (equivalent to  $1 \times 10^5$  cells/well). Add 50 ul supernatant produced by Example 11, 37°C for 48 to 72 hr. As a positive control, a growth factor known to activate PC12 cells through EGR can be used, such as 50 ng/ul of Neuronal Growth Factor (NGF). Over fifty-fold induction of SEAP is typically seen in the positive control wells. SEAP assay the supernatant according to Example 17.

#### **Example 16: High-Throughput Screening Assay for T-cell Activity**

NF- $\kappa$ B (Nuclear Factor  $\kappa$ B) is a transcription factor activated by a wide variety of agents including the inflammatory cytokines IL-1 and TNF, CD30 and CD40, lymphotoxin-alpha and lymphotoxin-beta, by exposure to LPS or thrombin, and by expression of certain viral gene products. As a transcription factor, NF- $\kappa$ B regulates the expression of genes involved in immune cell activation, control of apoptosis (NF- $\kappa$ B appears to shield cells from apoptosis), B and T-cell development, anti-viral and antimicrobial responses, and multiple stress responses.

In non-stimulated conditions, NF-  $\kappa$ B is retained in the cytoplasm with I- $\kappa$ B (Inhibitor  $\kappa$ B). However, upon stimulation, I-  $\kappa$ B is phosphorylated and degraded, causing NF-  $\kappa$ B to shuttle to the nucleus, thereby activating transcription of target genes. Target genes activated by NF-  $\kappa$ B include IL-2, IL-6, GM-CSF, ICAM-1 and class 1 MHC.

Due to its central role and ability to respond to a range of stimuli, reporter constructs utilizing the NF- $\kappa$ B promoter element are used to screen the supernatants produced in Example 11. Activators or inhibitors of NF- $\kappa$ B would be useful in treating



diseases. For example, inhibitors of NF- $\kappa$ B could be used to treat those diseases related to the acute or chronic activation of NF- $\kappa$ B, such as rheumatoid arthritis.

To construct a vector containing the NF- $\kappa$ B promoter element, a PCR based strategy is employed. The upstream primer contains four tandem copies of the NF- $\kappa$ B binding site (GGGGACTTTCCC) (SEQ ID NO:8), 18 bp of sequence complementary to the 5' end of the SV40 early promoter sequence, and is flanked with an XhoI site:  
 5':GCGGCCTCGAGGGGACTTTCCCGGGGACTTTCCGGGGACTTTCCGGGAC  
 TTTCCATCCTGCCATCTCAATTAG:3' (SEQ ID NO:9)

The downstream primer is complementary to the 3' end of the SV40 promoter and is flanked with a Hind III site: 5':GCGGCAAGCTTTTTGCAAAGCCTAGGC:3' (SEQ ID NO:4)

PCR amplification is performed using the SV40 promoter template present in the pB-gal:promoter plasmid obtained from Clontech. The resulting PCR fragment is digested with XhoI and Hind III and subcloned into BLSK2-. (Stratagene)  
 15 Sequencing with the T7 and T3 primers confirms the insert contains the following sequence:5':CTCGAGGGGACTTTCCCGGGGACTTTCCGGGGACTTTCCGGGA  
 CTTTCCATCTGCCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCCTAACTCC  
 GCCCATCCCGCCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGC  
 TGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGC  
 20 TATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAA  
 GCTT:3' (SEQ ID NO:10)

Next, replace the SV40 minimal promoter element present in the pSEAP2-promoter plasmid (Clontech) with this NF- $\kappa$ B/SV40 fragment using XhoI and HindIII.  
 25 However, this vector does not contain a neomycin resistance gene, and therefore, is not preferred for mammalian expression systems.

In order to generate stable mammalian cell lines, the NF- $\kappa$ B/SV40/SEAP cassette is removed from the above NF- $\kappa$ B/SEAP vector using restriction enzymes Sall and NotI, and inserted into a vector containing neomycin resistance. Particularly, the  
 30 NF- $\kappa$ B/SV40/SEAP cassette was inserted into pGFP-1 (Clontech), replacing the GFP gene, after restricting pGFP-1 with Sall and NotI.

Once NF- $\kappa$ B/SV40/SEAP/Neo vector is created, stable Jurkat T-cells are created and maintained according to the protocol described in Example 13. Similarly,

the method for assaying supernatants with these stable Jurkat T-cells is also described in Example 13. As a positive control, exogenous TNF alpha (0.1, 1, 10 ng) is added to wells H9, H10, and H11, with a 5-10 fold activation typically observed.

#### 5 **Example 17: Assay for SEAP Activity**

As a reporter molecule for the assays described in Examples 13-16, SEAP activity is assayed using the Tropix Phospho-light Kit (Cat. BP-400) according to the following general procedure. The Tropix Phospho-light Kit supplies the Dilution, Assay, and Reaction Buffers used below.

- 10 Prime a dispenser with the 2.5x Dilution Buffer and dispense 15 µl of 2.5x dilution buffer into Optiplates containing 35 µl of a supernatant. Seal the plates with a plastic sealer and incubate at 65°C for 30 min. Separate the Optiplates to avoid uneven heating.

- 15 Cool the samples to room temperature for 15 minutes. Empty the dispenser and prime with the Assay Buffer. Add 50 µl Assay Buffer and incubate at room temperature 5 min. Empty the dispenser and prime with the Reaction Buffer (see the table below). Add 50 µl Reaction Buffer and incubate at room temperature for 20 minutes. Since the intensity of the chemiluminescent signal is time dependent, and it takes about 10 minutes to read 5 plates on luminometer, one should treat 5 plates at each
- 20 time and start the second set 10 minutes later.

Read the relative light unit in the luminometer. Set H12 as blank, and print the results. An increase in chemiluminescence indicates reporter activity.

#### **Reaction Buffer Formulation:**

# of plates	Rxn buffer diluent (ml)	CSPD (ml)
10	60	3
11	65	3.25
12	70	3.5
13	75	3.75
14	80	4
15	85	4.25
16	90	4.5
17	95	4.75
18	100	5
19	105	5.25
20	110	5.5
21	115	5.75
22	120	6
23	125	6.25
24	130	6.5
25	135	6.75

26	140	7
27	145	7.25
28	150	7.5
29	155	7.75
30	160	8
31	165	8.25
32	170	8.5
33	175	8.75
34	180	9
35	185	9.25
36	190	9.5
37	195	9.75
38	200	10
39	205	10.25
40	210	10.5
41	215	10.75
42	220	11
43	225	11.25
44	230	11.5
45	235	11.75
46	240	12
47	245	12.25
48	250	12.5
49	255	12.75
50	260	13

**Example 18: High-Throughput Screening Assay Identifying Changes in Small Molecule Concentration and Membrane Permeability**

5 Binding of a ligand to a receptor is known to alter intracellular levels of small molecules, such as calcium, potassium, sodium, and pH, as well as alter membrane potential. These alterations can be measured in an assay to identify supernatants which bind to receptors of a particular cell. Although the following protocol describes an assay for calcium, this protocol can easily be modified to detect changes in potassium, sodium, pH, membrane potential, or any other small molecule which is detectable by a  
10 fluorescent probe.

The following assay uses Fluorometric Imaging Plate Reader ("FLIPR") to measure changes in fluorescent molecules (Molecular Probes) that bind small molecules. Clearly, any fluorescent molecule detecting a small molecule can be used instead of the calcium fluorescent molecule, fluo-3, used here.

15 For adherent cells, seed the cells at 10,000 -20,000 cells/well in a Co-star black 96-well plate with clear bottom. The plate is incubated in a CO<sub>2</sub> incubator for 20 hours. The adherent cells are washed two times in Biotek washer with 200 ul of HBSS (Hank's Balanced Salt Solution) leaving 100 ul of buffer after the final wash.

20 A stock solution of 1 mg/ml fluo-3 is made in 10% pluronic acid DMSO. To load the cells with fluo-3, 50 ul of 12 ug/ml fluo-3 is added to each well. The plate is

incubated at 37°C in a CO<sub>2</sub> incubator for 60 min. The plate is washed four times in the Biotek washer with HBSS leaving 100 ul of buffer.

For non-adherent cells, the cells are spun down from culture media. Cells are re-suspended to 2-5x10<sup>6</sup> cells/ml with HBSS in a 50-ml conical tube. 4 ul of 1 mg/ml fluo-3 solution in 10% pluronic acid DMSO is added to each ml of cell suspension. The tube is then placed in a 37°C water bath for 30-60 min. The cells are washed twice with HBSS, resuspended to 1x10<sup>6</sup> cells/ml, and dispensed into a microplate, 100 ul/well. The plate is centrifuged at 1000 rpm for 5 min. The plate is then washed once in Denley CellWash with 200 ul, followed by an aspiration step to 100 ul final volume.

For a non-cell based assay, each well contains a fluorescent molecule, such as fluo-3. The supernatant is added to the well, and a change in fluorescence is detected.

To measure the fluorescence of intracellular calcium, the FLIPR is set for the following parameters: (1) System gain is 300-800 mW; (2) Exposure time is 0.4 second; (3) Camera F/stop is F/2; (4) Excitation is 488 nm; (5) Emission is 530 nm; and (6) Sample addition is 50 ul. Increased emission at 530 nm indicates an extracellular signaling event which has resulted in an increase in the intracellular Ca<sup>++</sup> concentration.

#### **Example 19: High-Throughput Screening Assay Identifying Tyrosine Kinase Activity**

The Protein Tyrosine Kinases (PTK) represent a diverse group of transmembrane and cytoplasmic kinases. Within the Receptor Protein Tyrosine Kinase (RPTK) group are receptors for a range of mitogenic and metabolic growth factors including the PDGF, FGF, EGF, NGF, HGF and Insulin receptor subfamilies. In addition there are a large family of RPTKs for which the corresponding ligand is unknown. Ligands for RPTKs include mainly secreted small proteins, but also membrane-bound and extracellular matrix proteins.

Activation of RPTK by ligands involves ligand-mediated receptor dimerization, resulting in transphosphorylation of the receptor subunits and activation of the cytoplasmic tyrosine kinases. The cytoplasmic tyrosine kinases include receptor associated tyrosine kinases of the src-family (e.g., src, yes, lck, lyn, fyn) and non-receptor linked and cytosolic protein tyrosine kinases, such as the Jak family, members of which mediate signal transduction triggered by the cytokine superfamily of receptors (e.g., the Interleukins, Interferons, GM-CSF, and Leptin).

Because of the wide range of known factors capable of stimulating tyrosine kinase activity, the identification of novel human secreted proteins capable of activating

tyrosine kinase signal transduction pathways are of interest. Therefore, the following protocol is designed to identify those novel human secreted proteins capable of activating the tyrosine kinase signal transduction pathways.

Seed target cells (e.g., primary keratinocytes) at a density of approximately  
5 25,000 cells per well in a 96 well Loprodyne Silent Screen Plates purchased from Nalge Nunc (Naperville, IL). The plates are sterilized with two 30 minute rinses with 100% ethanol, rinsed with water and dried overnight. Some plates are coated for 2 hr with 100 ml of cell culture grade type I collagen (50 mg/ml), gelatin (2%) or polylysine (50 mg/ml), all of which can be purchased from Sigma Chemicals (St. Louis, MO) or  
10 10% Matrigel purchased from Becton Dickinson (Bedford, MA), or calf serum, rinsed with PBS and stored at 4°C. Cell growth on these plates is assayed by seeding 5,000 cells/well in growth medium and indirect quantitation of cell number through use of alamarBlue as described by the manufacturer Alamar Biosciences, Inc. (Sacramento, CA) after 48 hr. Falcon plate covers #3071 from Becton Dickinson (Bedford, MA) are  
15 used to cover the Loprodyne Silent Screen Plates. Falcon Microtest III cell culture plates can also be used in some proliferation experiments.

To prepare extracts, A431 cells are seeded onto the nylon membranes of Loprodyne plates (20,000/200ml/well) and cultured overnight in complete medium. Cells are quiesced by incubation in serum-free basal medium for 24 hr. After 5-20  
20 minutes treatment with EGF (60ng/ml) or 50 ul of the supernatant produced in Example 11, the medium was removed and 100 ml of extraction buffer ((20 mM HEPES pH 7.5, 0.15 M NaCl, 1% Triton X-100, 0.1% SDS, 2 mM Na<sub>3</sub>VO<sub>4</sub>, 2 mM Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> and a cocktail of protease inhibitors (# 1836170) obtained from Boehringer Mannheim (Indianapolis, IN) is added to each well and the plate is shaken on a rotating shaker for  
25 5 minutes at 4°C. The plate is then placed in a vacuum transfer manifold and the extract filtered through the 0.45 mm membrane bottoms of each well using house vacuum. Extracts are collected in a 96-well catch/assay plate in the bottom of the vacuum manifold and immediately placed on ice. To obtain extracts clarified by centrifugation, the content of each well, after detergent solubilization for 5 minutes, is removed and  
30 centrifuged for 15 minutes at 4°C at 16,000 x g.

Test the filtered extracts for levels of tyrosine kinase activity. Although many methods of detecting tyrosine kinase activity are known, one method is described here.

Generally, the tyrosine kinase activity of a supernatant is evaluated by determining its ability to phosphorylate a tyrosine residue on a specific substrate (a  
35 biotinylated peptide). Biotinylated peptides that can be used for this purpose include PSK1 (corresponding to amino acids 6-20 of the cell division kinase cdc2-p34) and

PSK2 (corresponding to amino acids 1-17 of gastrin). Both peptides are substrates for a range of tyrosine kinases and are available from Boehringer Mannheim.

The tyrosine kinase reaction is set up by adding the following components in order. First, add 10ul of 5uM Biotinylated Peptide, then 10ul ATP/Mg<sub>2+</sub> (5mM ATP/50mM MgCl<sub>2</sub>), then 10ul of 5x Assay Buffer (40mM imidazole hydrochloride, pH7.3, 40 mM beta-glycerophosphate, 1mM EGTA, 100mM MgCl<sub>2</sub>, 5 mM MnCl<sub>2</sub>, 0.5 mg/ml BSA), then 5ul of Sodium Vanadate(1mM), and then 5ul of water. Mix the components gently and preincubate the reaction mix at 30°C for 2 min. Initial the reaction by adding 10ul of the control enzyme or the filtered supernatant.

10 The tyrosine kinase assay reaction is then terminated by adding 10 ul of 120mM EDTA and place the reactions on ice.

Tyrosine kinase activity is determined by transferring 50 ul aliquot of reaction mixture to a microtiter plate (MTP) module and incubating at 37°C for 20 min. This allows the streptavidin coated 96 well plate to associate with the biotinylated peptide. Wash the MTP module with 300ul/well of PBS four times. Next add 75 ul of anti-phosphotyrosine antibody conjugated to horse radish peroxidase(anti-P-Tyr-POD(0.5u/ml)) to each well and incubate at 37°C for one hour. Wash the well as above.

20 Next add 100ul of peroxidase substrate solution (Boehringer Mannheim) and incubate at room temperature for at least 5 mins (up to 30 min). Measure the absorbance of the sample at 405 nm by using ELISA reader. The level of bound peroxidase activity is quantitated using an ELISA reader and reflects the level of tyrosine kinase activity.

#### 25 **Example 20: High-Throughput Screening Assay Identifying Phosphorylation Activity**

As a potential alternative and/or compliment to the assay of protein tyrosine kinase activity described in Example 19, an assay which detects activation (phosphorylation) of major intracellular signal transduction intermediates can also be used. For example, as described below one particular assay can detect tyrosine phosphorylation of the Erk-1 and Erk-2 kinases. However, phosphorylation of other molecules, such as Raf, JNK, p38 MAP, Map kinase kinase (MEK), MEK kinase, Src, Muscle specific kinase (MuSK), IRAK, Tec, and Janus, as well as any other phosphoserine, phosphotyrosine, or phosphothreonine molecule, can be detected by substituting these molecules for Erk-1 or Erk-2 in the following assay.

35

Specifically, assay plates are made by coating the wells of a 96-well ELISA plate with 0.1ml of protein G (1ug/ml) for 2 hr at room temp, (RT). The plates are then rinsed with PBS and blocked with 3% BSA/PBS for 1 hr at RT. The protein G plates are then treated with 2 commercial monoclonal antibodies (100ng/well) against Erk-1 and Erk-2 (1 hr at RT) (Santa Cruz Biotechnology). (To detect other molecules, this step can easily be modified by substituting a monoclonal antibody detecting any of the above described molecules.) After 3-5 rinses with PBS, the plates are stored at 4°C until use.

A431 cells are seeded at 20,000/well in a 96-well Loprodyne filterplate and cultured overnight in growth medium. The cells are then starved for 48 hr in basal medium (DMEM) and then treated with EGF (6ng/well) or 50 ul of the supernatants obtained in Example 11 for 5-20 minutes. The cells are then solubilized and extracts filtered directly into the assay plate.

After incubation with the extract for 1 hr at RT, the wells are again rinsed. As a positive control, a commercial preparation of MAP kinase (10ng/well) is used in place of A431 extract. Plates are then treated with a commercial polyclonal (rabbit) antibody (1ug/ml) which specifically recognizes the phosphorylated epitope of the Erk-1 and Erk-2 kinases (1 hr at RT). This antibody is biotinylated by standard procedures. The bound polyclonal antibody is then quantitated by successive incubations with Europium-streptavidin and Europium fluorescence enhancing reagent in the Wallac DELFIA instrument (time-resolved fluorescence). An increased fluorescent signal over background indicates a phosphorylation.

#### **Example 21: Method of Determining Alterations in a Gene Corresponding to a Polynucleotide**

RNA isolated from entire families or individual patients presenting with a phenotype of interest (such as a disease) is be isolated. cDNA is then generated from these RNA samples using protocols known in the art. (See, Sambrook.) The cDNA is then used as a template for PCR, employing primers surrounding regions of interest in SEQ ID NO:X. Suggested PCR conditions consist of 35 cycles at 95°C for 30 seconds; 60-120 seconds at 52-58°C; and 60-120 seconds at 70°C, using buffer solutions described in Sidransky, D., et al., Science 252:706 (1991).

PCR products are then sequenced using primers labeled at their 5' end with T4 polynucleotide kinase, employing SequiTherm Polymerase. (Epicentre Technologies). The intron-exon borders of selected exons is also determined and genomic PCR

products analyzed to confirm the results. PCR products harboring suspected mutations is then cloned and sequenced to validate the results of the direct sequencing.

PCR products is cloned into T-tailed vectors as described in Holton, T.A. and Graham, M.W., Nucleic Acids Research, 19:1156 (1991) and sequenced with T7 polymerase (United States Biochemical). Affected individuals are identified by mutations not present in unaffected individuals.

Genomic rearrangements are also observed as a method of determining alterations in a gene corresponding to a polynucleotide. Genomic clones isolated according to Example 2 are nick-translated with digoxigenin deoxy-uridine 5'-triphosphate (Boehringer Mannheim), and FISH performed as described in Johnson, Cg. et al., Methods Cell Biol. 35:73-99 (1991). Hybridization with the labeled probe is carried out using a vast excess of human cot-1 DNA for specific hybridization to the corresponding genomic locus.

Chromosomes are counterstained with 4,6-diamino-2-phenylidole and propidium iodide, producing a combination of C- and R-bands. Aligned images for precise mapping are obtained using a triple-band filter set (Chroma Technology, Brattleboro, VT) in combination with a cooled charge-coupled device camera (Photometrics, Tucson, AZ) and variable excitation wavelength filters. (Johnson, Cv. et al., Genet. Anal. Tech. Appl., 8:75 (1991).) Image collection, analysis and chromosomal fractional length measurements are performed using the ISee Graphical Program System. (Inovision Corporation, Durham, NC.) Chromosome alterations of the genomic region hybridized by the probe are identified as insertions, deletions, and translocations. These alterations are used as a diagnostic marker for an associated disease.

25

**Example 22: Method of Detecting Abnormal Levels of a Polypeptide in a Biological Sample**

A polypeptide of the present invention can be detected in a biological sample, and if an increased or decreased level of the polypeptide is detected, this polypeptide is a marker for a particular phenotype. Methods of detection are numerous, and thus, it is understood that one skilled in the art can modify the following assay to fit their particular needs.

For example, antibody-sandwich ELISAs are used to detect polypeptides in a sample, preferably a biological sample. Wells of a microtiter plate are coated with specific antibodies, at a final concentration of 0.2 to 10 ug/ml. The antibodies are either monoclonal or polyclonal and are produced by the method described in Example 10.

35



The wells are blocked so that non-specific binding of the polypeptide to the well is reduced.

The coated wells are then incubated for > 2 hours at RT with a sample containing the polypeptide. Preferably, serial dilutions of the sample should be used to validate results. The plates are then washed three times with deionized or distilled water to remove unbounded polypeptide.

Next, 50 ul of specific antibody-alkaline phosphatase conjugate, at a concentration of 25-400 ng, is added and incubated for 2 hours at room temperature. The plates are again washed three times with deionized or distilled water to remove unbounded conjugate.

Add 75 ul of 4-methylumbelliferyl phosphate (MUP) or p-nitrophenyl phosphate (NPP) substrate solution to each well and incubate 1 hour at room temperature. Measure the reaction by a microtiter plate reader. Prepare a standard curve, using serial dilutions of a control sample, and plot polypeptide concentration on the X-axis (log scale) and fluorescence or absorbance of the Y-axis (linear scale). Interpolate the concentration of the polypeptide in the sample using the standard curve.

### **Example 23: Formulating a Polypeptide**

The secreted polypeptide composition will be formulated and dosed in a fashion consistent with good medical practice, taking into account the clinical condition of the individual patient (especially the side effects of treatment with the secreted polypeptide alone), the site of delivery, the method of administration, the scheduling of administration, and other factors known to practitioners. The "effective amount" for purposes herein is thus determined by such considerations.

As a general proposition, the total pharmaceutically effective amount of secreted polypeptide administered parenterally per dose will be in the range of about 1 µg/kg/day to 10 mg/kg/day of patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.01 mg/kg/day, and most preferably for humans between about 0.01 and 1 mg/kg/day for the hormone. If given continuously, the secreted polypeptide is typically administered at a dose rate of about 1 µg/kg/hour to about 50 µg/kg/hour, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be employed. The length of treatment needed to observe changes and the interval following treatment for responses to occur appears to vary depending on the desired effect.

Pharmaceutical compositions containing the secreted protein of the invention are administered orally, rectally, parenterally, intracisternally, intravaginally,

intraperitoneally, topically (as by powders, ointments, gels, drops or transdermal patch), buccally, or as an oral or nasal spray. "Pharmaceutically acceptable carrier" refers to a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

The secreted polypeptide is also suitably administered by sustained-release systems. Suitable examples of sustained-release compositions include semi-permeable polymer matrices in the form of shaped articles, e.g., films, or microcapsules. Sustained-release matrices include polylactides (U.S. Pat. No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman, U. et al., Biopolymers 22:547-556 (1983)), poly (2-hydroxyethyl methacrylate) (R. Langer et al., J. Biomed. Mater. Res. 15:167-277 (1981), and R. Langer, Chem. Tech. 12:98-105 (1982)), ethylene vinyl acetate (R. Langer et al.) or poly-D-(-)-3-hydroxybutyric acid (EP 133,988). Sustained-release compositions also include liposomally entrapped polypeptides. Liposomes containing the secreted polypeptide are prepared by methods known per se: DE 3,218,121; Epstein et al., Proc. Natl. Acad. Sci. USA 82:3688-3692 (1985); Hwang et al., Proc. Natl. Acad. Sci. USA 77:4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese Pat. Appl. 83-118008; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily, the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. percent cholesterol, the selected proportion being adjusted for the optimal secreted polypeptide therapy.

For parenteral administration, in one embodiment, the secreted polypeptide is formulated generally by mixing it at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a pharmaceutically acceptable carrier, i.e., one that is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation. For example, the formulation preferably does not include oxidizing agents and other compounds that are known to be deleterious to polypeptides.

Generally, the formulations are prepared by contacting the polypeptide uniformly and intimately with liquid carriers or finely divided solid carriers or both. Then, if necessary, the product is shaped into the desired formulation. Preferably the carrier is a parenteral carrier, more preferably a solution that is isotonic with the blood of the recipient. Examples of such carrier vehicles include water, saline, Ringer's solution, and dextrose solution. Non-aqueous vehicles such as fixed oils and ethyl oleate are also useful herein, as well as liposomes.

The carrier suitably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability. Such materials are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) polypeptides, e.g., polyarginine or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids, such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, manose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counterions such as sodium; and/or nonionic surfactants such as polysorbates, poloxamers, or PEG.

The secreted polypeptide is typically formulated in such vehicles at a concentration of about 0.1 mg/ml to 100 mg/ml, preferably 1-10 mg/ml, at a pH of about 3 to 8. It will be understood that the use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of polypeptide salts.

Any polypeptide to be used for therapeutic administration can be sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). Therapeutic polypeptide compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

Polypeptides ordinarily will be stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous solution or as a lyophilized formulation for reconstitution. As an example of a lyophilized formulation, 10-ml vials are filled with 5 ml of sterile-filtered 1% (w/v) aqueous polypeptide solution, and the resulting mixture is lyophilized. The infusion solution is prepared by reconstituting the lyophilized polypeptide using bacteriostatic Water-for-Injection.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the polypeptides of the present invention may be employed in conjunction with other therapeutic compounds.

35

**Example 24: Method of Treating Decreased Levels of the Polypeptide**

It will be appreciated that conditions caused by a decrease in the standard or normal expression level of a secreted protein in an individual can be treated by administering the polypeptide of the present invention, preferably in the secreted form.

- 5 Thus, the invention also provides a method of treatment of an individual in need of an increased level of the polypeptide comprising administering to such an individual a pharmaceutical composition comprising an amount of the polypeptide to increase the activity level of the polypeptide in such an individual.

- 10 For example, a patient with decreased levels of a polypeptide receives a daily dose 0.1-100 ug/kg of the polypeptide for six consecutive days. Preferably, the polypeptide is in the secreted form. The exact details of the dosing scheme, based on administration and formulation, are provided in Example 23.

**Example 25: Method of Treating Increased Levels of the Polypeptide**

- 15 Antisense technology is used to inhibit production of a polypeptide of the present invention. This technology is one example of a method of decreasing levels of a polypeptide, preferably a secreted form, due to a variety of etiologies, such as cancer.

- 20 For example, a patient diagnosed with abnormally increased levels of a polypeptide is administered intravenously antisense polynucleotides at 0.5, 1.0, 1.5, 2.0 and 3.0 mg/kg day for 21 days. This treatment is repeated after a 7-day rest period if the treatment was well tolerated. The formulation of the antisense polynucleotide is provided in Example 23.

**Example 26: Method of Treatment Using Gene Therapy**

- 25 One method of gene therapy transplants fibroblasts, which are capable of expressing a polypeptide, onto a patient. Generally, fibroblasts are obtained from a subject by skin biopsy. The resulting tissue is placed in tissue-culture medium and separated into small pieces. Small chunks of the tissue are placed on a wet surface of a tissue culture flask, approximately ten pieces are placed in each flask. The flask is  
30 turned upside down, closed tight and left at room temperature over night. After 24 hours at room temperature, the flask is inverted and the chunks of tissue remain fixed to the bottom of the flask and fresh media (e.g., Ham's F12 media, with 10% FBS, penicillin and streptomycin) is added. The flasks are then incubated at 37°C for approximately one week.

At this time, fresh media is added and subsequently changed every several days. After an additional two weeks in culture, a monolayer of fibroblasts emerge. The monolayer is trypsinized and scaled into larger flasks.

5 pMV-7 (Kirschmeier, P.T. et al., DNA, 7:219-25 (1988)), flanked by the long terminal repeats of the Moloney murine sarcoma virus, is digested with EcoRI and HindIII and subsequently treated with calf intestinal phosphatase. The linear vector is fractionated on agarose gel and purified, using glass beads.

The cDNA encoding a polypeptide of the present invention can be amplified using PCR primers which correspond to the 5' and 3' end sequences respectively as set  
10 forth in Example 1. Preferably, the 5' primer contains an EcoRI site and the 3' primer includes a HindIII site. Equal quantities of the Moloney murine sarcoma virus linear backbone and the amplified EcoRI and HindIII fragment are added together, in the presence of T4 DNA ligase. The resulting mixture is maintained under conditions appropriate for ligation of the two fragments. The ligation mixture is then used to  
15 transform bacteria HB101, which are then plated onto agar containing kanamycin for the purpose of confirming that the vector has the gene of interest properly inserted.

The amphotropic pA317 or GP+am12 packaging cells are grown in tissue culture to confluent density in Dulbecco's Modified Eagles Medium (DMEM) with 10% calf serum (CS), penicillin and streptomycin. The MSV vector containing the gene is  
20 then added to the media and the packaging cells transduced with the vector. The packaging cells now produce infectious viral particles containing the gene (the packaging cells are now referred to as producer cells).

Fresh media is added to the transduced producer cells, and subsequently, the media is harvested from a 10 cm plate of confluent producer cells. The spent media,  
25 containing the infectious viral particles, is filtered through a millipore filter to remove detached producer cells and this media is then used to infect fibroblast cells. Media is removed from a sub-confluent plate of fibroblasts and quickly replaced with the media from the producer cells. This media is removed and replaced with fresh media. If the titer of virus is high, then virtually all fibroblasts will be infected and no selection is  
30 required. If the titer is very low, then it is necessary to use a retroviral vector that has a selectable marker, such as neo or his. Once the fibroblasts have been efficiently infected, the fibroblasts are analyzed to determine whether protein is produced.

The engineered fibroblasts are then transplanted onto the host, either alone or after having been grown to confluence on cytodex 3 microcarrier beads.

It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples. Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

- 5       The entire disclosure of each document cited (including patents, patent applications, journal articles, abstracts, laboratory manuals, books, or other disclosures) in the Background of the Invention, Detailed Description, and Examples is hereby incorporated herein by reference.

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- 5 (i) APPLICANTS: Human Genome Sciences, Inc. et al.
- (ii) TITLE OF INVENTION: 20 Human Secreted Proteins
- 10 (iii) NUMBER OF SEQUENCES: 61
- (iv) CORRESPONDENCE ADDRESS:
- (A) ADDRESSEE: Human Genome Sciences, Inc.
- (B) STREET: 9410 Key West Avenue
- 15 (C) CITY: Rockville
- (D) STATE: Maryland
- (E) COUNTRY: USA
- (F) ZIP: 20850
- 20 (v) COMPUTER READABLE FORM:
- (A) MEDIUM TYPE: Diskette, 3.50 inch, 1.4Mb storage
- (B) COMPUTER: HP Vectra 486/33
- (C) OPERATING SYSTEM: MSDOS version 6.2
- 25 (D) SOFTWARE: ASCII Text
- (vi) CURRENT APPLICATION DATA:
- (A) APPLICATION NUMBER:
- 30 (B) FILING DATE: April 07, 1998
- (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
- (A) APPLICATION NUMBER:
- 35 (B) FILING DATE:
- (viii) ATTORNEY/AGENT INFORMATION:
- (A) NAME: A. Anders Brookes
- 40 (B) REGISTRATION NUMBER: 36,373
- (C) REFERENCE/DOCKET NUMBER: P2005PCT
- (vi) TELECOMMUNICATION INFORMATION:
- 45 (A) TELEPHONE: (301) 309-8504
- (B) TELEFAX: (301) 309-8439

## (2) INFORMATION FOR SEQ ID NO: 1:

- 50 (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 733 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- 55 (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

60 GGGATCCGGA GCCCAAATCT TCTGACAAAA CTCACATG CCCACCGTGC CCAGCACCTG 60

AATTCGAGGG TGCACCGTCA GTCTTCCTCT TCCCCCAAA ACCCAAGGAC ACCCTCATGA 120  
 TCTCCCGGAC TCCTGAGGTC ACATGCGTGG TGGTGGACGT AAGCCACGAA GACCCTGAGG 180  
 5 TCAAGTTCAA CTGGTACGTG GACGGCGTGG AGGTGCATAA TGCCAAGACA AAGCCGCGGG 240  
 AGGAGCAGTA CAACAGCACG TACCGTGTGG TCAGCGTCCT CACCGTCCTG CACCAGGACT 300  
 GGCTGAATGG CAAGGAGTAC AAGTGCAAGG TCTCCAACAA AGCCCTCCCA ACCCCCATCG 360  
 10 AGAAAACCAT CTCCAAGCC AAAGGGCAGC CCCGAGAACC ACAGGTGTAC ACCCTGCCCC 420  
 CATCCCGGGA TGAGCTGACC AAGAACCAGG TCAGCCTGAC CTGCCTGGTC AAAGGCTTCT 480  
 15 ATCCAAGCGA CATCGCCGTG GAGTGGGAGA GCAATGGGCA GCCGGAGAAC AACTACAAGA 540  
 CCACGCCTCC CGTGCTGGAC TCCGACGGCT CCTTCTTCCT CTACAGCAAG CTCACCGTGG 600  
 ACAAGAGCAG GTGGCAGCAG GGGAACTCT TCTCATGCTC CGTGATGCAT GAGGCTCTGC 660  
 20 ACAACCACTA CAGCAGAAG AGCCTCTCCC TGTCTCGGG TAAATGAGTG CGACGGCCGC 720  
 GACTCTAGAG GAT 733

25

## (2) INFORMATION FOR SEQ ID NO: 2:

30

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

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## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Trp Ser Xaa Trp Ser  
 1 5

40

## (2) INFORMATION FOR SEQ ID NO: 3:

45

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 86 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

50

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

GCGCCTCGAG ATTTCCCCGA AATCTAGATT TCCCCGAAAT GATTCCCCG AAATGATTTC 60  
 55 CCCGAAATAT CTGCCATCTC AATTAG 86

60

## (2) INFORMATION FOR SEQ ID NO: 4:



- 5 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 27 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

10 GCGGCAAGCT TTTTGCAAAG CCTAGGC 27

- 15 (2) INFORMATION FOR SEQ ID NO: 5:

- 20 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 271 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

25 CTCGAGATTT CCCCGAAATC TAGATTTCCT CGAAATGATT TCCCCGAAAT GATTTCCTCCG 60  
AAATATCTGC CATCTCAATT AGTCAGCAAC CATAGTCCCG CCCCTAACTC CGCCCATCCC 120  
GCCCCTAACT CCGCCAGTT CCGCCCATTC TCCGCCCAT GGCTGACTAA TTTTTTTTAT 180  
30 TTATGCAGAG GCCGAGGCCG CCTCGGCTC TGAGCTATTC CAGAAGTAGT GAGGAGGCTT 240  
TTTTGGAGGC CTAGGCTTTT GCAAAAAGCT T 271

35

- (2) INFORMATION FOR SEQ ID NO: 6:

- 40 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 32 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear

45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

GCGCTCGAGG GATGACAGCG ATAGAACCCC GG 32

50

- (2) INFORMATION FOR SEQ ID NO: 7:

- 55 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 31 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear

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100

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

5 GCGAAGCTTC GCGACTCCCC GGATCCGCCT C 31

10 (2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 12 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

20 GGGGACTTTC CC 12

25 (2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 73 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

35 GCGGCCTCGA GGGGACTTTC CCGGGGACTT TCCGGGGACT TTCCGGGACT TTCCATCCTG 60  
 CCATCTCAAT TAG 73

40

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 256 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

45

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

50 CTCGAGGGGA CTTTCCCGGG GACTTTCCGG GGACTTTCCG GGACTTTCCA TCTGCCATCT 60  
 CAATTAGTCA GCAACCATAG TCCCGCCCCT AACTCCGCCC ATCCCGCCCC TAACTCCGCC 120  
 55 CAGTTCGGCC CATTCTCCGC CCCATGGCTG ACTAATTTT TTTATTTATG CAGAGGCCGA 180  
 GGCCGCCTCG GCCTCTGAGC TATTCCAGAA GTAGTGAGGA GGCTTTTITG GAGGCCTAGG 240  
 60 CTTTTCGAAA AAGCTT 256

## (2) INFORMATION FOR SEQ ID NO: 11:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 919 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

5  
15  
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45

AATTCCGGCAG AGGTCCAATT CAGTTTTTCA GGCCAGTGCA NCTTGATACC AAAACCAATA 60  
AAACAAACAA ACAACAAAA AACATAAGC TATAGACCA AGTCTCATAG ATTTAGATGC 120  
AAAATCCTAA AATTGAAAA AAAAGTCTAG TCATATCCAT AACTGTATC ATCACCAGA 180  
GATGTTTATT AGGGCAATCA AAAGATGATT TATTATTTT TAAAAATCA ATGTGGCCTT 240  
CCCTTCCTCT TTCTTTTGAT TCCCTCTTT GAGTTTTAT GTGTCTCTT TGCCTTCCT 300  
TCCCAGAGTG GAGGAGTTAG ACCTGCATTG TGGGATGAGA GGAGTTGTGG CTATGTGTCT 360  
GCTGGCACCA AGAGGGCTGA GGGTGAGTG TGAAGGGAC AGGGGGAGGA GATGGGCAGC 420  
ATTGTTAAGA GATTGGTACC ACTGAGCAA TATGTTGAGA ATGATGATGG CAAGGTTTCT 480  
CCCTGTTAGA GAAGGTATTT GTAGAAATAG GAATGAGGAG AGCTAGAAA CCTGGAGTGT 540  
GGGATTAGAA TAGAACTCAT ATCTTTTAAA TACATAGGAA CAATAGAGAA ATTGTTGGGT 600  
GTGCCCATAT ACATATATTT TGTGATTCAT TCTACCGAGA GGACATAAAT GCAGTCACAG 660  
CTCAGTAACA GTAAACACAC CAACTGCCAA GTTATTATTT CCTAAATACT ATCCACAAAA 720  
AAGGGGACCA GGGATGATTC CTAGTCGGAG ATTGGGAGAA AAAGAAGATG AGCCTGAATC 780  
ATTTTCATGTA CCTAACAGAA AGAAAATACT CTGGCTGGG TCAGTGGCTC ATGTTTGTAA 840  
TTCTAGCATG TTAGGARGTC SARGTGGTG TGTGCTTGA GCCCAGGART TTGAGACCAG 900  
CCCAGGCAAC ATGGCAATA 919

## (2) INFORMATION FOR SEQ ID NO: 12:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1026 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

60

GGCAGGAGT TTTGTCTGCA GCACCTGCCA TGAACCTCTG GTTGACATGA TTTATTTTTC 60

102

	GAAGAATGAG AAGCTATACT GTGGCAGACA TTA CTGTGAC AGCGAGAAAC CCCGATGTGC	120
	TGGCTGTGAC GAGCTGATAT TCAGCAATGA GTATACCCAG GCAGAAAACC AGAATTGGCA	180
5	CCTGAAACAC TTC TGTCTGCT TTGACTGTGA TAGCATTCTA GCTGGGGAGA TATACGTGAT	240
	GGTCAATGAC AAGCCCGTGT GCAAGCCCTG CTATGTGAAG AATCACGCTG TGGTGTGTCA	300
	AGGATGCCAC AATGCCATCG ACCCAGAAGT GCAGCGCGTG ACCTATAACA ATTT CAGCTG	360
10	GCATGCATCC ACAGAGTGCT TTCTGTGCTC TTGCTGCAGC AAATGCCTCA TTGGGCAGAA	420
	GTT CATGCCA GTAGAAGGGA TGGTTTCTG TTCAGTGGAA TGT AAGAAGA GGATGTCTTA	480
15	GGAGGAGGGC ACCCAGAAGT ATCGAGCCAT AGCTATCCAA AGTGGTCTGC ATTTCTACTG	540
	TAAAATGCAA TTTGAAAAA ATAAAACGCA AAAAAAGAAA CTGTAAAGGA AACCAAGAGA	600
	TTTTGTTTAA TTTTTTTGGC CATTTTTTCT TCATCAATTT TTTTTCGGTC TCAACTTTTA	660
20	AACTTGGTTT AAGCATTGTA TTGTAAAAC AGTAAATAAT TGTATCTTTC CATAGCTTTT	720
	CAAATGTGAA ATCATTTTGT GAAGCTTGA TCTCATTA AA CTTCATGTCT CTATTCATT	780
25	TGTGCCACAC ACTTAAAAGT TAGTGTACTG AATGGAAAGA TGAGCATTC TAGTCTACA	840
	CTTCTTTTTT CCCCTCATG TGTAAAATGA AAAGAAACT AAATTTGCCC TAATACCAAG	900
	GCGCTACGTT TATTCCTCG TCTTATTCAC TGACCTTTTG TAATGATACA CAGTGAATTC	960
30	TTTTTTGACA AAGNGGAATG CGGTTTGGTA TGCAGAGCTG CTGGTTTTAA NGCCCATGGC	1020
	ATTNAC	1026
35		

## (2) INFORMATION FOR SEQ ID NO: 13:

- 40 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 2067 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear
- 45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

	AATTCGGCAC GAGCTTTTAA TAGGAAGTAA TGTCTCACC AAGAGAAATG AAGAGCAGGG	60
50	AAGAGTGA CT TTCTCCTTCT CCTCCCTCT CCCCTGGATA TGGAAC TCA CCATTATGCA	120
	CTGCTTCITT TTGTGGTTGC TGCTTTTGG ACTTCTTGA ATTAGTGGTT TCCTTGTTA	180
	TATTT CAGTG GCTGGTARCA GTATATATGT CATGTGGAAG GTGGARAAGG AAATGAATAC	240
55	TTAGGTCTCA AAGACCCACT CTCCATGGCT GCTTTAGCAG ATGGCTGTTT CTTTCTCTCC	300
	CTTG CAGGTT GGGGATAGGA TTGTCACCAT CTGTGGCACA TCCACTGAGG GCATGACTCA	360
60	CACCCAAGCA GTTAACCTAC TGAAAAATGC ATCTGGCTCC ATTGAAATGC AGGTGGTTGC	420

	TGGAGGAGAC GTGAGTGTGG TCACAGGTCA TCAGCAGGAG CCTGCAAGTT CCAGTCTTTC	480
	TTTCACTGGG CTGACGTCAA GCAGTATATT TCAGGATGAT TTAGGACCTC CTCAATGTAA	540
5	GTCTATTACA CTAGAGCGAG GACCAGATGG CTTAGGCTTC AGTATAGTTG GGAGGATATG	600
	GGCAGCCCTC ATGGGAGACT TACCCATTTA TGTTAAAACA GTGTTTTGCA AAGGGGAGCA	660
10	GCCTCTGGAA GACGGACGTC TTGAAAAGGG GGGCGATTCC AGATCATGTC TGTCAATGGG	720
	CAGAGTCTAG AWGGAGTCAC CCATGAAGAA GCTKTTGCCA TCCTTAAACG GACAAAAGGG	780
	CACCTGCACT TTGATGGTTC TCTCTGAAT TGGCTGCCAG AATTGAACCA ACCCAACCCC	840
15	TAGCTCACCT CCTACTGTAA AGAGAATGCA CTGGTCCTGA CAATTTTAT GCTGTGTTCA	900
	GCCGGTCTT CAAACTGTA GGGGGAAAT AACACTTAAG TTTCTTTTTC TCATCTAGAA	960
20	ATGCTTTCCT TACTGACAAC CTAACATCAT TTTTCTTTTC TTCTTGCAAT TTGTGAACCT	1020
	AAAGAGAAGG AATATTTGTG TAGGTGAATC TCGTTTTTAT TTGTGGAGAT ATCTAATGTT	1080
	TTGTAGTCAC ATGGGCAAGA ATTATTACAT GCTAAGCTGG TTAGTATAAA GAAAGATAAT	1140
25	TCTAAAGCTA ACCAAAGAAA ATGGCTTCAG TAAATTAGGA TGAAAATGA AAATATAAAA	1200
	TAAAGAAGAA AATCTCGGGG AGTTTAAAAA AAATGCCTCA ATTTGGCAAT CTACCTCCTC	1260
30	TCCCCACCCC AAACATAAAA AAAAAAAGG GGTTTTCTAA TGAAAATCTT TAAAAATACT	1320
	GTCAGTATTT TAAAATTTTC AACAGTATTA TAAAAACATT GCATCTCCCC ACCTCTAATA	1380
	TGCATATATA TTTTTCCTGC TAAAATTGGT TTCTACAATT GAGTAAATGG CAAATACATG	1440
35	AAGCAATGTC CCTAAATTTT ATAAAGAAAT TATATTTAAT GCACATTTC AATTTTCATT	1500
	TTATTTTGA CCTTTTGTA AATATTTTCA TGTGCTATA AGTAAATGAT GATGCCACCC	1560
40	CAGTTGACT ATGGKTTTTC TAGAAAGCAA CTATGCTGCT AACCATAGAG GAACATAGAA	1620
	GGGTTCCAGA ATCTTTAGTG CTGGTTTTAA CAACCGATGC AACATTAAAA ATGTGTTAGT	1680
	GTGCTGTGCA ATTGGTTTTT AATTCATATT AATCTTAATG ACAGAGAACA ATGTGTTACT	1740
45	AATTATTTG GTTGATGCC ATTAGTAAAT TGATAGAAAA ATTAAGGGGA TTAACATAAC	1800
	TTCATTTCAT TGCCTTATAT TAACATCTTA TAATACAATA GTTTAAGACT AAGGGAACA	1860
50	GATGGAGCTG TTTATTGAGA CAACTGGTGA GGAATTATCA TGTGTTTCATT CCCATTTTAG	1920
	AGCGTGAAAC TCCTACATTA GAATATATAA AGTCACTTTA AATATCTATA TTTGTAACAG	1980
	AAGTAGTGA CAGATATTTT ATTACAGCAT TTTTGTGTAA ATGCAGAATT AAAGTGAATA	2040
55	AATAAGAATT TTCAGTGGTG CACAAAT	2067
60		

## (2) INFORMATION FOR SEQ ID NO: 14:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1341 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

5	GGCACGAGCT GGCCTGGACG AACGGGAAGC CGGGAGCTCG GCCACGGGTG GCGAGGCTGC	60
	GGTGAGGCCT GGTCTCCGGC TGCCAGACCA TGCTGAGTGG AGCACGCTGC AGGCTCGCCT	120
15	CAGCGCTGCG GGAACGCGC GCGCCGCCGT CCGCGGTCGC CCGTAGTGCC TGCACGCGTC	180
	GGGGTCGCGG CCTTTGGCAC CGGGGCAAGA AGACTGAGGA GCCGCCCCGC GACTTCGATC	240
20	CGGCGCTGCT GGAGTTCCTG GTGTGCCCGC TCTCCAAGAA GCCGCTCAGA TATGAAGCAT	300
	CAACAAACGA ATTGATTAAT GAAGAGTGG GAATAGCTTA TCCAATCATT GATGGGATCC	360
	CTAATATGAT ACCACAGGCA GCTAGGATGA CACGTCAAAG TAAGAAGCAA GAAGAAGTGG	420
25	AGCAGCGCTA GTTCATAATT TAAAAAATT AAAAAACGC AACAGCCAAC TTTTCTTAAT	480
	ACCATATACC TTTTAAACA CAGTGGCAGG TAATAAGTGG AAGAGAAGAA TGTTTCTGTC	540
30	TCTTCTACG TTGACTGTTT TTATCCACT GGTTCCTTTA GCAGGACTGT TCTACTCAGC	600
	CTCTGTGAA GAAACTTCC CACAGGGCTG CACTAGCACA GCCAGCCTTT GCTTTTACAG	660
	CCTGCTCTTG CCTATTACCA TACCAGTGA TGTATCTTC CACCTTTGGA CTGGATGGG	720
35	TATTAACTC TTCAGGCATA ATTGATGCAA CTAGAGTCAA TATGCTGTAT ATATTAATGA	780
	TAGCTCTTGG GCATCGATCT CTGAAAGCTC AAATGGATGG AATTAGTTT GCGGGAAGA	840
40	GGCTTTGCTT TGCGCATATC AGGCTTAGGA CTGTGGGAGG CTTAAGTTGC AGATGCTTCT	900
	TTTATGTAC TCTTGTCTG CCCTGTGTTT TTGAAGGCTC TGACTTATAA CTGCTGTATC	960
	AGAAGAAACA TTTTGACAGT GTCTTGGTTG GAGATGAACA TCCCTAATG ACATGTGATG	1020
45	ACTATTTCTT ATTCCATTCA TCTAAGAGTC ATTGAAATT TGTTTTGCTT GTTTGTTTAG	1080
	CTTCAAGGTC TTTGGTAAAG TCACATGTTA AGGATGACTG AAATAATTCC AAAGGAGTGA	1140
50	TGTTGGAATA GTCCCTCTAA GGGAGAGAAA TGCATTTGAA CGAATGTGAT ATAAAACCAC	1200
	ATAATCAAT AGAACTTCA TGTACTTACA AAACTGAGT TTGTAAATT ACCTTCATTT	1260
	CTTTGACATT AAATGCTTAT ATTAGCAATA AACATGTTGA CACTTTCCTA TAAAAAWAA	1320
55	AAAAAAAAA AAAAAAAAT T	1341

## 60 (2) INFORMATION FOR SEQ ID NO: 15:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1443 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

5

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

10	GGCCGGGCGC TCCTGAAGCA GCAGTTATGG AGCTTCCCTC AGGGCCGGGG CCGGAGCGGC	60
	TCTTTGACTC GCACCGGCTT CCGGGTGACT GCTTCCTACT GCTCGTGCTG CTGCTCTACG	120
	CGCCAGTCGG GTTCTGCCTC CTCGTCTGCG GCCTCTTTCT CGGGATCCAC GTCTTCCTGG	180
15	TCAGCTGCGC GCTGCCAGAC AGCGTCCTTC GCAGATTCTG AGTGGCGACC ATGTGTGCGG	240
	TGCTAGGGCT CGTGGCCCGG CAGGAGGACT CCGGACTCCG GGATCACAGT GTCAGGGTCC	300
20	TCATTTCCAA CCATGTGACA CCTTTCGACC ACAACATAGT CAATTTGCTT ACCACCTGTA	360
	GCACCCCTCT ACTCAATAGT CCCCCAGCT TTGTGTGCTG GTCTCGGGGC TTCATGGAGA	420
	TGAATGGGCG GGGGAGTTG GTGGAGTCAC TCAAGAGATT CTGTGCTTCC ACGAGGCTTC	480
25	CCCCCACTCC TCTGCTGCTA TTCCCTGAGG AAGAGGCCAC CAATGGCCCG GAGGGGCTCC	540
	TGCGCTTCAG TTCTGGCCA TTCTCTATCC AAGATGTGGT ACAACCTCTT ACCCTGCAAG	600
30	TTCAGAGACC CCTGGTCTCT GTGACGGTGT CAGATGCCTC CTGGGTCTCA GAACTGCTGT	660
	GGTCACTTTT CGTCCCTTTC ACGGTGTATC AAGTAAGGTG GCTTCGTCTT GTTCATCGCC	720
	AACTAGGGGA AGCGAATGAG GAGTTTGAC TCCGTGTACA ACAGCTGGTG GCCAAGGAAT	780
35	TGGGCCAGAC AGGGACACGG CTCACTCCAG CTGACAAAGC AGAGCACATG AAGCGACAAA	840
	GACACCCGAC ATTGCGCCCC CAGTCAGCCC AGTCTTCTTT CCCTCCCTCC CCTGGTCTTT	900
40	CTCCTGATGT GCAACTGGCA ACTCTGGCTC AGAGAGTCAA GGAAGTTTGT CCCCATGTGC	960
	CATTGGGTGT CATCCAGAGA GACCTGGCCA AGACTGGCTG TGTAGACTTG ACTATCACTA	1020
	ATCTGCTTGA GGGGGCCGTA GCTTTCATGC CTGAAGACAT CACCAAGGGA ACTCAGTCCC	1080
45	TACCCACAGC CTCTGCCTCC AAGTTTCCCA GCTCTGGCCC GGTGACCCCT CAGCCAACAG	1140
	CCCTAACATT TGCCAAGTCT TCCTGGGCCC GGCAGGAGAG CCTGCAGGAG CGCAAGCAAG	1200
50	CACTATATGA ATACGCAAGA AGGAGATTCA CAGAGAGACG AGCCCAGGAG GCTGACTGAG	1260
	CTCAAAGGAA CAGGATGGCA CCCAGAGCCG CAGGACGGAG ACTGGGGGCA GCCCTCACCC	1320
	AACTCACAAC AGGCTGGATG GGTGGTGGT AAAAAGGGAA GGATGAGGCT CCCCAATGT	1380
55	CACATTAAAT TCATGGTTTT CATTCAAAAA AAAAAAAAAA AAACCTCCCG GGGGNGGCCC	1440
	CGT	1443

60

## (2) INFORMATION FOR SEQ ID NO: 16:

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## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 654 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

10

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

GCGAATTCAT TTCCGANCTG AGGAAGCAAA AAACCCCGCC AACAATGTCA TTCCACCCAC 60  
 15 CAGTGCCACA ATGGGTCAGC TGTACCAGGA ACACCATGAA GAAGACTTCT TTCTCTACAT 120  
 TGCCTACAGT GACGAAAGTG TCTACGGTCT GTGAAGCTGC TGCCCTGAG CTGGAGGGGG 180  
 GTCTCATTCT ACAAAGAGAG AGGTGGCCCC CCTTCTTGA CCTCCTCCTC CTTCAAGCTC 240  
 20 AAACACCACC TCCCTTATTC AGGACCGGCA CTTCTTAATG TTTGTGGCTT TCTCTCCAGC 300  
 CTCTCTTAGG AGGGGTAATG GTGGAGTTGG CATCTGTAA CTCTCCTTTC TCCTTTCTTC 360  
 25 CCGTTTCTCT GCCCGCCTTT CCCATCCTGC TGTAGACTTC TTGATTGTCA GTCTGTGTCA 420  
 CATCCAGTGA TTGTTTGGT TTCTGTCCC TTTCTGACTG CCAAGGGGC TCAGAACCCC 480  
 AGCAATCCCT TCCTTTCACT ACCTTCTTTT TTGGGGGTAG TTGAAGGGA CTGAAATTGT 540  
 30 GGGGGGAAGG TAGGAGGCAC ATCAATAAAG AGGAAACCAC CAAGCTGAAA AAAAAAAAAA 600  
 AAAAAGCTGA GGGGGGGCCC GGTACCCATT GGCCCTAAGG GGGGGGNTTA NANT 654

35

## (2) INFORMATION FOR SEQ ID NO: 17:

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## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 749 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

45

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

AAAGATGGCT GCGCCAGGTA ATTTGAGCAA AGGCCACAGT GAACTCCGGC GTGGCTGAGG 60  
 50 AAGGAGGAGG CACCCACAGG CTGCTGGGAG GAGAGCATAA GGCTCAAAAT GGAAAATCAT 120  
 AAATCCAATA ATAAGGAAAA CATAACAATT GTTGATATAT CCAGAAAAAT TAACCAGCTT 180  
 CCAGAAGCAG AAAGGAATCT ACTTGAAAAT GGATCGGTTT ATGTTGGATT AAATGCTGCT 240  
 55 CTTTGTGGCC TCATAGCAA CAGTCTTTTT CGACGCATCT TGAATGTGAC AAAGGCTCGC 300  
 ATAGCTGCTG GCTTACCAAT GGCAGGATA CCTTTCTTA CAACAGACTT AACTTACAGA 360  
 60 TGTTTGTGTA GTTTTCCTTT GAATACAGGT GATTGAGATT GTGAAACCTG TACCATAACA 420



CGGAGTGGAC TGACTGGTCT TGTTATTGGT GGTCTATACC CTGTTTCTT GGCTATACCT 480  
 GTAAATGGTG GTCTAGCAGC CAGGTATCAA TCAGCTCTGT TACCACACAA AGGGAACATC 540  
 5 TTAAGTTACT GGATTAGAAC TTCTAAGCCT GTCTTTAGAA AGATGTTATT TCCTATTTTG 600  
 CTCCAGACTA TGTTTTGAGC ATACCTTGGG TCTGAACAAT ATAACTACT TATAAAGGCC 660  
 10 CTTCAGTTAT CTGAACCTGG CAAAGAAATT CACTGATTTT AAACAAATAT GTAAACAAAA 720  
 ATAAAATGGT AAAACARAA AAAAAAAAAA 749

15

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:  
 20 (A) LENGTH: 511 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

AATTGCGCAC GAGCCAGATT CCCATAAGC ACATGGTCTA ATCTGTTACG TAACAGCAAG 60  
 ACAGCGTCAC CTCACCTGTT CTCGCCCTCA AATGGGAACG CTGGCCTGGG ACTAAAGCAT 120  
 30 AGACCACCAG GCTGAGTATC CTGACCTGAG TCATCCCCAG GGATCAGGAG CCTCCAGCAG 180  
 GGAACCTTCC ATTATATTCT TCAAGCAACT TACAGCTGCA CCGACAGTTG CGATGAAAGT 240  
 35 TCTAATCTCT TCCCTCCTCC TGTGCTGCC ACTAATGCTG ATGTCCATGG TCTCTAGCAG 300  
 CCTGAATCCA GGGGTGCGCA GAGGCCACAG GGACCGAGGC CAGGTTCTA GGAGATGGCT 360  
 CCAGGAAGGC GGCCAAGAAT GTRAGTGCAA AGATTGGTTC CTGAGAGCCC GAGAAGAAAA 420  
 40 TTCATGACAG TGTCTGGGCT GCCAAGAAR CARTGCCCNT GTGATCTTTT CAAGGGCATG 480  
 TGAAGAAAAC AAGNCACCAA AGGCACCACA G 511

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(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:  
 50 (A) LENGTH: 689 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

55

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

GGCAGGAGGA GCGATGGCC CACGGGGCTG CTAGCCGTGC TGCGGCCCCCT GCTCACCTGC 60  
 60 CGGCCCCCTGC AAGGCACGAC GCTGCAACGG GATGTGCTGC TCTTTGAGCA TGATCGGGGC 120

CGCTTCTTCA CCATCCTCGG GCTGTTCTGC GCGGGCCAGG GCGTCTTCTG GCCTTCCATG 180  
 GCTGTGGCAG CCGTGTCCCG GCGCCCGGTT CCGGTGCAGC CTCTGGATGC GGAGGTCCCA 240  
 5 AATCGTGGCC CCTTCGACCT GCGCTCCGCG YTCTGGCGCT NACGGTCTGG CCGTCGGCTG 300  
 CGGCGCCATC GGAGCCCTCG TACTCGGTGC TGGTCTTCTC TTCTCTCTCC GGTCTGTGGC 360  
 10 CTCAGTGGTG CTTCGAGCTG GAGGGCAGCA GGTGACCCTC ACCACTCATG CCCCCTTTGG 420  
 CTTGGGGGCC CATTTACAG TTCTTTGAA GCAGGTATCT TGCATGGCCC ACCGGGGTGA 480  
 AGTCCCTGCC ATGCTACCTC TGAARKCAA AGCCGACGC TTCTATTTC TCTTGGACAA 540  
 15 AACTGGACAC TTCCCYTAAC ACAAACCTYT TTGACAATAC TGTGGGTGCC TACCGGAGCT 600  
 TGTGAAGAAA TGACCTCAAG TCACTCACCT CTCCAAGAGG AGGATAAAAA CTGAACCTYG 660  
 20 GGGAGCCAGG TGTGTGGTT CACACCTGT 689

25 (2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1147 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

35 TCGACCCACG CGTCCGGGCG GGCCTGTTTC CCGGAGGCGC GTGGGGCTTG AGGCCGAGAA 60  
 CGGCNTTGC TGCCACCAAC ATGGAGACTT TGTACCGTGT CCGGTTCCTTA GTGCTCGAAT 120  
 GTCCCAACCT GAAGCTGAAG AAGCCGCCCT GGTGACAT GCGTCGGCC ATGACTGTGT 180  
 40 ATGCTCTGGT GGTGGTGTCT TACTTCCTCA TCACCGGAGG AATAATTAT GATGTTATTG 240  
 TTGAACCTCC AAGTGTCCGT TCTATGACTG ATGAACATGG GCATCAGAGG CCAGTAGCTT 300  
 45 TCTTGGCCTA CAGAGTAAAT GGACAATATA TTATGGAAGG ACTTGATCC AGCTTCCTAT 360  
 TTACAATGGG AGGTTTAGGT TTCATAATCC TGGACCGATC GAATGCACCA AATATCCCAA 420  
 AACTCAATAG ATTCCCTCTT CTGTTTATTG GATTCGTCTG TGTCTATTG AGTTTTTTCA 480  
 50 TGGCTAGAGT ATTCATGAGA ATGAACTGC CCGGCTATCT GATGGGTTAG AGTGCCTTTG 540  
 AGAAGAAATC AGTGATACT GGATTTGCTC CTGTCAATGA AGTTTTAAAG GCTGTACCAA 600  
 55 TCCTCTAATA TGAAATGTGG AAAAGAATGA AGAGCAGCAG TAAAAGAAAT ATCTAGTGAA 660  
 AAAACAGGAA GCGTATTGAA GCTTGGACTA GAATTTCTTC TTGGTATTAA AGAGACAAGT 720  
 60 TTATCACAGA ATTTTTTTTC CTGCTGGCCT ATTGCTATAC CAATGATGTT GAGTGGCATT 780

TTCTTTT TAG TTTTCATTA AAATATATTC CATATCTACA ACTATAATAT CAAATAAAGT 840  
 GATTATTTTT TACAACCCCTC TTAACATTTT TTGGAGATGA CATTTCTGAT TTTCAGAAAT 900  
 5 TAACATAAAA TCCAGAAGCA AGATTCCGTA AGCTGAGAAC TCTGGACAGT TGATCAGCTT 960  
 TACCTATGGT GCTTTGCCTT TAACTAGAGT GTGTGATGGT AGATTATTTC AGATATGTAT 1020  
 GTAAACTGT TTCTGAACA ATAAGATGTA TGAACGGAGC AGAAATAAAT ACTTTTCTTA 1080  
 10 ATTAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA 1140  
 AAAAAANN 1147

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(2) INFORMATION FOR SEQ ID NO: 21:

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- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 532 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

CTTGTCTC TTTCTCTAC ACCAGCAGTT TTTATTGCC TGTTCCCAG AGTAATGTGG 60  
 30 GCCATGGAGT CAGGCCACCT CCTCTGGGCT CTGCTGTCA TGCAGTCCTT GTGGCCTCAA 120  
 CTGACTGATG GAGCCACTCG AGTCTACTAC CTGGCATCC GGGATGTGCA GTGGAACTAT 180  
 GCTCCCAAGG GAAGAAATGT CATCAGAAC CAGCCTCTGG ACAGTGACAT GTAGGTTTAA 240  
 35 TTTCTTGGG TATTTGAGGG GAAGTTATGG GAGCACTCTT GAGGTCAGGA AGTAGCCTCT 300  
 TGAGGCCCT TCCCARGGT GTGGTAGCAG CCAGCTCTG ATTGCTCCGA GCTGTACATA 360  
 40 CTCAGTGGCA GATTTCTGG GAAGAAGCTA GTGAGTCAG AASCCAGCAT TTCATCTGGA 420  
 GTTGSCGTA ACATTTT TAG AGTCTAAAG ARAATTCCAT ATTTGCTGTT TTCTAATCTC 480  
 ATACCCACAA TGCTACTTAT TTAATAACAA CTGTTTGACT TTAAAAAAA AA 532  
 45

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(2) INFORMATION FOR SEQ ID NO: 22:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 2743 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear

55

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

TGAACCCGCC CCTCTCCAG AGTGGAGCTG CGGGGGCGG GAACAGGCAC GGAGAAAATA 60  
 60

	AACAAGACTA AAAAGTCCTG AGTAGCGCTG TGTGGCGCA AACCTGAACC CACCTTTTGC	120
	ACCACGCGGG ACCCGGCACT CTTCTTGCCA CCCACCCCTG AGAGGGTGCG CGGCCGACCC	180
5	CAGTACTAGA AAACACTCGT CACCTCACTC AAGACGGTA CGAAGGCCAA CGGACGCCTT	240
	CCTTTAGAAC GCTCAGCACA CAGAGCAACT TCTCAGCCT ACTCTCAAAT GCGTACTCC	300
	AAACTAGCAC TCCGACGTC CAGCTGTGAA CCCAGAGCG CGGAAAGCCC TGGAACCCAG	360
10	CGCCCGGGCA TGYGCAGACG CGTTGTGTG GTGGGCGTGG CTCCTCCCG ACCCGGCGCC	420
	CCGGCCTGCC GCCCGTGTG CGCATGCGG ACTGAGCCGG GTGGATGGTA CTGCTGCATC	480
15	CGGGTGTCTG GAGGCTGTGG CCGTTTGTG TTCTTGGCTA AAATCGGGG AGTGAGCGG	540
	GCCGGCGCGG CGCGACACG GGCTCCGAA CCACTGCACG ACGGGGCTGG ACTGACCTGA	600
	AAAAAATGTC TGGATTTCTA GAGGGCTTGA GATGCTCAGA ATGCATTGAC TGGGGGAAA	660
20	AGCGCAATAC TATTGCTTCC ATTGCTGCTG GTGTACTATT TTTTACAGGC TGGTGGATTA	720
	TCATAGATGC AGCTGTTATT TATCCACCA TGAAAGATT CAACCACTCA TACCATGCCT	780
25	GTGGTGTAT AGCAACCATA GCCTTCCTAA TGATTAATGC AGTATCGAAT GGACAAGTCC	840
	GAGGTGATAG TTACAGTGAA GGTGTCTGG GTCAAACAGG TGCTCGCATT TGGCTTTTCG	900
	TTGGTTTCAT GTTGGCCTTT GGATCTCTGA TTGCATCTAT GTGGATTCTT TTTGGAGGTT	960
30	ATGTTGCTAA AGAAAAGAC ATAGTATACC CTGGAATTGC TGTATTTTTC CAGAATGCCT	1020
	TCATCTTTT TGGAGGGCTG GTTTTAAST TTGGCCGAC TGAAGACTTA TGGCAGTGAA	1080
35	CACATCTGAT TTCCACAGC ACAACAGCCC TGCATGGGTT TGTGTGTTT TTTACTGCTC	1140
	ACTCCCAACC TTTTGTAAAG CCATTTTCTA AACTTATTTT TGAGTGTAGT CTCAGCTTAA	1200
	AGTTGTGTAA TACTAAATC ACGAGAACAC CTAACAACA ACCAAAAATC TATTGTGGTA	1260
40	TGCACCTGAT TAACTTATAA AATGTTAGAG GAACTTTCA CATGAATAAT TTTGTCAAA	1320
	TTTTATCATG GTATAATTG TAAAAATAA AAGAAATTAC AAAAGAAATT ATGGATTTGT	1380
45	CAATGTAAGT ATTTGTCATA TCTGAGGTCC AAAACCACAA TGAAAGTGCT CTGAAGATTT	1440
	AATGTGTTTA TTCAATGTG GTCTCTCTG TGTCAAATGT TAAATGAAAT ATAAACATTT	1500
	TTTAGTTTTT AAAATATTCC GTGGTCAAAA TTCTTCCTCA CTATAATTGG TATTTACTTT	1560
50	TACCAAAAAT TCTGTGAACA TGTAAATGTA CTGGCTTTTG AGGGTCTCCC AAGGGGTGAG	1620
	TGGACGTGTT GGAAGAGAGA AGCACCATGG TCCAGCCACC AGGCTCCCTG TGTCCCTTCC	1680
55	ATGGGAAGGT CTTCCGCTGT GCCTCTCATT CCAAGGGCAG GAAGATGTGA CTCAGCCATG	1740
	ACACGTGGT CTGGTGGGAT GCACAGTCAC TCCACATCCA CCATTGAAGG AAAGGAAAAA	1800
60	AGGGCAGAGA CTTGACACTC CAGTCTTAGA CAGGGGACAA TTTCTTTGTA GTTGTCTGA	1860

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TAATAAACTG GGTTCATGT TGCATTTTCAT CTGTGTAAGT AACCCTAGC CTAATAGACC 1920  
 AGCACGGTCA ACTAGAATGA CAAACATTAG CTACTGGGAA CTCTTGTGT CTCTTCCTCT 1980  
 5 TCAGAACTCT TGTCCCCCA GGCTCCCCAC CTCTGCAAGA TGGGATACAC TCTTCAGGAC 2040  
 CACAGTTTGA AATGTCTTTC ATAAAAATGT CTACAATTAG CCTTCGGAAT ATTGCTGGG 2100  
 TGATTTAAAT GTGTGGGTCT CATTTCCATG CTAGCCATGG TCATCTGACA GTCTCTACRC 2160  
 10 TGTGAATATT GCCTGGTGAT CAAGACTCTC CTCAAAGAAA TGACTTGCTG TCATCCCACA 2220  
 TGAACCTCTG ATGTTTTTTC TACAAAAGTC CATAAAATGT GAAAACCTGA GAAGATCTTA 2280  
 15 GAGGTGAAG CCCACCTTCT CTTTTCACAT AGGAGGGAAC AGACCATGGA AATTAAACG 2340  
 ACTTCCTCAC GGTACAGAA CTAGTTTTTT AATCCTCAGG CAGTGCATCC CCCACCCCTA 2400  
 CAACTGAGCA CAACCTCTTT CCCCACAGTG CAATTCAGAA TATGCTCAGG GAATGCCAGC 2460  
 20 CACCTTGTA AACTGCTGGG AGAAAAGCAT GATTCCCACA AGGACTAAGT ATCAGTGATT 2520  
 TGTAAATTTT CTGTTTGTGA TTATCTGCTT TGCTGATGTA GACAAGAGTT AACTGAGTAG 2580  
 25 CATGCTTTAT TAAGCATGAG AAAGAATCTT AAGAATTGTC AATAAAATTA ACCCAAACT 2640  
 TTAATAATGT GTCTGTAACC AAGAAAATAT TGATAGCATC ATCCTAATGA AACTAAACAT 2700  
 TTATTTTAAA CTTATTAAAT TGACTCTTAA ACTAAAAAAA AAA 2743  
 30

(2) INFORMATION FOR SEQ ID NO: 23:

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(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 820 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

AATTCGGCAC GAGATCACAA GCTACTAGCG CTTCATTAA CAATTCAAAT CCATCTACCT 60  
 45 CTGAGCAGGC CTCTAATACT RCTTCAGCTG TCACCAGTAG CCAACCTTCC ACAGTGTGAG 120  
 AGACTTCAGC TACTCTTACA AGCAATAGTA CCACTGGCAC TTCTATAGGA GATGACTCAA 180  
 50 GGAGAACTAC ATCTAGTGCT GTAACGGAAA CTGGCCCTCC TGCAATGCCA AGGTTACCTT 240  
 CCTGCTGTCC CCAGCACTCA CCATGTGGAG GGTGTCACA GAACCACCAT GCATTAGGAC 300  
 ATCCTCATAC AAGTTGCTTT CAGCAGCATG GTCACCATT TCAACATCAT CACCACCACC 360  
 55 ACCATACTCC CCACTCAGAC CGCCGCGCG CCGCCATCAT GGACACCAGC CGTGTGCAGC 420  
 CTATCAAGCT GGCCAGGGTC ACCAAGGTCC TGGGAGGAC CGGTCTCAG GGACAGTGCA 480  
 60 CGCAGGTGCG CGTGAATTC ATGACGACA CGAGCCGATC CATCATCCGC AATGTAAAAG 540

5 G C C C C G T G C G C G A G G G C G A C G T G C T C A C C C T T T T G G A G T C A G A G C G A G A A G C C C G G A G G T 600  
 T G C G C T G A G C T T G G C T G C T C G C T G G T C T T G G A T G T C G G G T T C G A C C A C T T G G C C G A T G G 660  
 G A A T G G T C T G T C A C A R T C T G C T C C T T T T T T T G T C C G C C A C A C G T A A C T G A G A T G C T C C T 720  
 T T A A A T A A A G C G T T T G T G T T T C A A G T T A A A A A A A A A A A A A A A A A C Y C C G G G G 780  
 10 G G G N C C C G G T A C C C A T T G G C C T W T A G T G G G T C G T T T T T C 820

15 (2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:

20 (A) LENGTH: 995 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

25 A T T A T G A A T T C G G A C G C A G A G G T G A C A G T G G A G C T G G C C T G G G C C A G G A C G G C A G G T G G 60  
 C C C T G G A G A T G G G A A A G T G T C T G T G C G A G C G C G T G A G C T C T C T C T C T G T T T C T C C T T T T 120  
 T T C C T C T A C T C C T T C C C C T T C A C A C C C C C G T G G C T G G A A G G A A C C T C G G C T T C C C T G A A A 180  
 30 G C T T G G G G G T C C C A C C C T T C T T A C C C C A C C C G G G A G G A A C G C C A G G G C C C G G G C T T G T 240  
 T T C T C C T C T T G T T T C C T T T T G G G C A G T T T G A T C A C T G A T C G A G T A A G G A A T G A C C T T T A 300  
 35 G A T T G T G C G A C T T T T G T T T T T G T T T T T T T A A A T T T T T T A A A C C A A G A A T G A T T T C T C C T 360  
 G C T T C C T T C T C C T C A C C A T C T T C C C A G A C G G A G T T C A A A G G C C A C T T C T C A A G C A G C T T T 420  
 T G G C A C C T T C A G C C T C A G A G T G A A T C T T T T A A A G A C A G G A C C C C T A T G T C C A G G A A A G G 480  
 40 G G A A A A G G A A C T T T G C C A A T G A T A G T G A C C A C A G C A A A A G C A A T A A T A A T A A T A T T A A T 540  
 A A T A A T A A A G A G A A T A A A A T A A T A A A A A T A A A A A C A A T A G C A C A G C C C T T G T T G A G G T C 600  
 45 A G C A G G G A G G A G G G G C T G C C C G G A G T T G G G T C C T T G C C T G G A T T T G A C A C A C A C T T C 660  
 C T G T A G T G A G C A C T T T G T A T G A A T C G T G G A C T T C C T G T T C T C A A G G C G C A G G T A T T T A T T 720  
 C T G T A T C T G T C T A G A G C A C A C A C A A A A T C C A A C C T T C T A A T A A C A T G A T G G C G C A G T C 780  
 50 C C A C T C C C T G C C T G C C C T G T N C C C C T A T C C C C C C A G G C C T G G G A T C T T C A G G N G T C G G T 840  
 G T G G G G A G G G G C C C C T G C C C T C C T T G C C T T G A T T T G C T C C C T G G G T C C A G C T G G T T C C 900  
 55 A G G C C T G T G A A T G T C A G T T C G T C G G G C A C T G A C T C C G T C T G C T C T T G G G C C T T G G G G T C A 960  
 T T T G A C A A A T A T T T G C C C A A G G G C T C C C A A G N C C A 995

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## (2) INFORMATION FOR SEQ ID NO: 25:

## (i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 649 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear

## 10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

GAATTCGGCA CGAGCACTTT AAGCACTATT CATTTTCCCT AATTTYTTCC ACTCACCGGG 60  
AATTATTCAT TGTTCTCCTT CACTCCCTTG CTCAGACATG TKTCCCATGT CACCCCAGGG 120  
15 GAGGCTGTYA TGTCAACAAGA TGTGTTACT GATGATCTTG GAATTWTTTC CCTGCCCCCA 180  
GCCTGGGACT AATGTAATTA TTATTTCAAT GIGCTTTTTC TTAAGCCATA GCAATGCAAT 240  
20 TTATCTTCAA CATTATCATT TTAAACATC TGTAAATTAT TAACAATTTA CTGCTVCTYC 300  
TTGCACAAAA TGCTATTCCA GTAACATTTA TTAATTAAGT TATGTWCACA TACCAAAGAT 360  
TTTACAGGCT TGTAAAATAG CAGGCCATTY CAAGGATTTC TCTCTGGTA RAGAMATTTG 420  
25 TWGGGAAAGA GTTATATAAT CACTAAATTA CATTATATC AAGAACACTT TTCTTGAGTG 480  
AAATTAGTCT AGGTTTGCTT AAGTGTCTCT TTTTATTTA ACTAAGNAAA TATCATGCCA 540  
30 TATCTGTCTT ATATTGCTAT TATCTCTCCC TTCCGAGGAC CACATCTTCT GTTACAAGAG 600  
GGAGACTGTG CTTCAAGGGA GTAGAAGAGA TGGTTTCAGT ATATTTTNA 649

35

## (2) INFORMATION FOR SEQ ID NO: 26:

## (i) SEQUENCE CHARACTERISTICS:

- 40 (A) LENGTH: 979 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear

## 45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

AGTTTCTTTG CCTTTCCTTG TAAATTTTAG AATAACCTTG TCTATATCTA CAGATCTTGC 60  
TGGGATTTTG ATAAGAATTG CATTGATCCT TTATACCAAT TTGAGGAGGA TTTATGTAAT 120  
50 TTTTGTGCGAG TCTTTTAATC CATGAACATG TTATATCTGT TATATTTAGC ATTTTGATTT 180  
TTTCTCAGT GTTGCATAGT TTTCAGTATA CAAATCCTGT ACCCTTTTTT TTTAGATTTA 240  
55 CACCTAGTAC TTTATTTTTT GAGCAATTGT AAATGGTATT GTATTTTAAA TTTCATTGCC 300  
CATGTGTTC ATTGCTAATA TACTGAAATA ARATTGGCTT TTGTATGTTT ATCTTGTATC 360  
CCACAATCTT GCTGAACCTM CTTGTTCTAA RACTTTTGT ARATTACGGG GAATTCYAC 420  
60

ATARACAGTT ATGTCATCTG CAAATGGGGA TAGTTTTGTT TTTTCCTTTA CAAACTGTAT 480  
 ACTTTTTTTA TCTGTTTCTT GCTTTATTGC GAGAACTTCT AGAGCTGTGT TGAGTAATAG 540  
 5 TGGATATCTT TGCCTTGTTT ATGTTCTTAT AGGGAAAGCA TTCAGTCTTT CACCATTTAG 600  
 TATAATGTTA GCTGTAGGGA CTTTTTAGAT CCCTTTACCA GATTGATGAA AGTTCTCTCTC 660  
 TATTCCCAT TTTCTGAGAG GCTTTTAAAA AAAAGAATGA ATGGGTGTTT GATTTTGTC 720  
 10 AATGCATTTC TGTGTCACTT GCTATGAACA NGTGTTTTTC TTATTTAGCC TGTTAATATG 780  
 GTAGATTACA TTGATTGGCT TTCTGATCTT GAGTCAGCAT TGCATCCTTG GAATAAACTC 840  
 15 CACCTGGGTG TGGAGAANAA ATCTTTTTTT TTTTTTTTTT TTTANGAGAT GGAGTCTCGC 900  
 TCTGTCAACC AGGCTGGAGT GCAGTGGTGT GATTGTGGCT CACGGCAAGT TCCTCTCTCT 960  
 GGGTCCCAT CATTCTCAA 979  
 20

25 (2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 905 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

CGACGGTTC ACCTTGTTGG CCAGGCTGGT TTTGTTGGCC AATTGTCTCT AACTGCTGT 60  
 35 CAAAAAAGG AATGGATCAG ATTGTCTTGA ATAGGCAGAG GCTAACCTGT AATCACCTGT 120  
 GTGATGAGAA ASAGCTTTGA CTGCATTTTA CTCTGACCT GGCCTAAGCT TTCTGTTTAC 180  
 40 ATAAGATTTT TCAAGAATTC AACTTCAAGT AGCAGCCGAG AGAGCTGCCT CAGGATTCTC 240  
 TCAAAACTG GGAATAATAT GGGAACATTT GTTCTTCTA AAAATAAGGC AAATGTTACA 300  
 TTGAATGATT TGGGGGGTGA GGTTTAATTG GAAATGGTCT CTGGGGACTG AAAACTGATG 360  
 45 TTTTTCAGAG TTACCTCAGG GAAACGGAGG TTTGTTGAGT TTACAGACAC ATTAAACCAA 420  
 AGGCCGTGGG AAAACCCCTC TCCAGCTCCA GGGGATTGGT CAGGACCACC CACTAACCAG 480  
 50 TGCCTTCCTT CTTAACATTG ACTTTTAGCA GCTTGTGTTT ATTTTACATG GGCAGTTTGT 540  
 ATGGGAAATT GCCATGACCA CAGGGGTTTG GAGTCTGCT TTTTTTTTTT CTCTTCTTTT 600  
 TTCGGGGGAC TGGGGGACTC CTCCAAGAT CACATTTTAG CATCTTCTC TCCTACTCCA 660  
 55 TTTAGAAAAA TAAGTAACAG GTGAAATGTG GTCTCAGTGT TAACGGGATA ATTCTGCTAC 720  
 CGGCTCTCC CTGATGATTC TGAAATACAC TACTGAACGA GCTCTGGCTG GTCCTTCTA 780  
 60 TCCTGGATGT GGTCTTCTG TGTAGCAATT CCTTGATGTC CAGTTTGGAA AGATGTACTC 840



TTCTCAACAA GAAAACTTA AATCCGTCGT GCCCAAAAAA AAAAAAAAAA AAAAAAAAAA 900  
CTCGA 905

5

(2) INFORMATION FOR SEQ ID NO: 28:

10

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 299 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

GAATTCCTTT TTTTTTTTTT TTTTTTTGAC AGAGTCTCAC TGTGTCTCCC AGGCTGGAGT 60  
GCAGCAGTAC AATCTCGGCT CTCTGCAACA TCTTCCTTCC AGGTTCAAGC GATTCTCATG 120  
CCTCAGCCCC ACAAGTAGCT GGGATTACAG GCATGCATCA CCACACCCTG CTAATTTTTG 180  
TATTTTTAGT AGAGACGGCG TTTCACCATC TTGGCCAGAC TGGTCTCAA CTCCTGGCCT 240  
CAAGTGATTC GTCTGCCTCA GCCTCCCAA AAAAAAAAAA AAAAAAAAAA AAGAATTCC 299

30

(2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 338 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

35

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

GAATTCCTCT TTTTTTTTTT TTTTTTTTTT GAGACAGGG TCTTCCTCTG TTGCTCAGGC 60  
TAAAGTGAG TGGTGCAATC ATAGCTCACT GTGGCCTCTA CTTCTTGGTC TCAAGTGATC 120  
ATCYTCCCTC AGCYTCCTAA GTACCTAGGA GTCATGCACC AACATGCCCA GCTAAGTATT 180  
TTATTTTTGG TAGAGATAAG GTCTTGCTGT GTTGGCCAGG CTAGTCTCAA ATTCTTGGCC 240  
TCAAGCAATC CTTCTGCCTT GGCCTCCCAA ATTGTTGGGT TTKACAGGCA TTAGCCKTTA 300  
TGCTTGGSCC CCAGGTCCTT TTTTTTTAAA ANNTNAAA 338

55

(2) INFORMATION FOR SEQ ID NO: 30:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 500 base pairs

60

116

(B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

GAATTCCTTTT TTTTCTTCAGT ACCCACTACA GCATTATCCT TGTCTGCAGG 60  
 TTTGAGGATG GATCTGCTCC CAGGGAGGGC GAGACAAGTG CACCTAGGTT GCCGGAAGTT 120  
 10 GTACGCATCA CTTCTGCTGG TATCTGTTAG CCCAACCCAG GCCACGTGGA CTCTCCCAGA 180  
 TGCCAGAGAC CATGAGAAGA AGAAGAGAAA GGGCTTGGAG AAGGATGGAC CACTCACCAT 240  
 15 TTGCTGGAAT AAACAATACT GCAGTCCTTG TTTTAACACT TCATTTTCAT CATGCCACAC 300  
 TGTGCGTAAC TGAATAACGG CCACCCACAG ATGTCAGGGC TTATCCCTGG AACCCGGAAA 360  
 TGGTGTGTTGC AGATGGGAGT GAAAGCAGGT CTTTGAYAGAA GGGATCAAGT TAGGGATCTT 420  
 20 GAGATGGGAA GAATTTCTCTG GATTGTCTCTG CTAGACACTA AAAAAAAAAA AAAAAAAAAA 480  
 GAATTCAAAA AGCTTCTCGA 500

25

(2) INFORMATION FOR SEQ ID NO: 31:

30 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 94 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

Met Ile Tyr Tyr Phe Leu Lys Asn Gln Cys Gly Leu Pro Phe Leu Phe  
 1 5 10 15  
 Leu Leu Ile Pro Leu Phe Glu Phe Leu Cys Val Ser Phe Ala Phe Pro  
 20 25 30  
 Ser Gln Ser Gly Gly Val Arg Pro Ala Leu Trp Asp Glu Arg Ser Cys  
 35 40 45  
 45 Gly Tyr Val Ser Ala Gly Thr Lys Arg Ala Glu Gly Glu Val Trp Lys  
 50 55 60  
 Gly Gln Gly Glu Glu Met Gly Ser Ile Val Lys Arg Leu Val Pro Leu  
 65 70 75 80  
 50 Ser Lys Tyr Val Glu Asn Asp Asp Gly Lys Val Ser Pro Cys  
 85 90

55

(2) INFORMATION FOR SEQ ID NO: 32:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23 amino acids  
 (B) TYPE: amino acid

60

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

5 Met His Pro Gln Ser Ala Phe Cys Ala Leu Ala Ala Ala Asn Ala Ser  
 1 5 10 15  
 Leu Gly Arg Ser Ser Cys Gln  
 20

10

(2) INFORMATION FOR SEQ ID NO: 33:

(i) SEQUENCE CHARACTERISTICS:

15

(A) LENGTH: 42 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

20 Met His Cys Phe Phe Leu Trp Leu Leu Leu Phe Gly Leu Leu Gly Ile  
 1 5 10 15  
 Ser Gly Phe Leu Gly Tyr Ile Ser Val Ala Gly Xaa Ser Ile Tyr Val  
 20 25 30  
 25 Met Trp Lys Val Glu Lys Glu Met Asn Thr  
 35 40

30

(2) INFORMATION FOR SEQ ID NO: 34:

(i) SEQUENCE CHARACTERISTICS:

35

(A) LENGTH: 71 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

40 Met Phe Leu Ser Leu Pro Thr Leu Thr Val Leu Ile Pro Leu Val Ser  
 1 5 10 15  
 Leu Ala Gly Leu Phe Tyr Ser Ala Ser Val Glu Glu Asn Phe Pro Gln  
 20 25 30  
 45 Gly Cys Thr Ser Thr Ala Ser Leu Cys Phe Tyr Ser Leu Leu Pro  
 35 40 45  
 Ile Thr Ile Pro Val Tyr Val Phe Phe His Leu Trp Thr Trp Met Gly  
 50 55 60  
 50 Ile Lys Leu Phe Arg His Asn  
 65 70

55

(2) INFORMATION FOR SEQ ID NO: 35:

(i) SEQUENCE CHARACTERISTICS:

60

(A) LENGTH: 410 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

5 Met Glu Leu Pro Ser Gly Pro Gly Pro Glu Arg Leu Phe Asp Ser His  
 1 5 10 15  
 Arg Leu Pro Gly Asp Cys Phe Leu Leu Leu Val Leu Leu Leu Tyr Ala  
 20 25 30  
 10 Pro Val Gly Phe Cys Leu Leu Val Leu Arg Leu Phe Leu Gly Ile His  
 35 40 45  
 Val Phe Leu Val Ser Cys Ala Leu Pro Asp Ser Val Leu Arg Arg Phe  
 50 55 60  
 15 Val Val Arg Thr Met Cys Ala Val Leu Gly Leu Val Ala Arg Gln Glu  
 65 70 75 80  
 Asp Ser Gly Leu Arg Asp His Ser Val Arg Val Leu Ile Ser Asn His  
 85 90 95  
 Val Thr Pro Phe Asp His Asn Ile Val Asn Leu Leu Thr Thr Cys Ser  
 100 105 110  
 25 Thr Pro Leu Leu Asn Ser Pro Pro Ser Phe Val Cys Trp Ser Arg Gly  
 115 120 125  
 Phe Met Glu Met Asn Gly Arg Gly Glu Leu Val Glu Ser Leu Lys Arg  
 130 135 140  
 30 Phe Cys Ala Ser Thr Arg Leu Pro Pro Thr Pro Leu Leu Leu Phe Pro  
 145 150 155 160  
 Glu Glu Glu Ala Thr Asn Gly Arg Glu Gly Leu Leu Arg Phe Ser Ser  
 165 170 175  
 35 Trp Pro Phe Ser Ile Gln Asp Val Val Gln Pro Leu Thr Leu Gln Val  
 180 185 190  
 40 Gln Arg Pro Leu Val Ser Val Thr Val Ser Asp Ala Ser Trp Val Ser  
 195 200 205  
 Glu Leu Leu Trp Ser Leu Phe Val Pro Phe Thr Val Tyr Gln Val Arg  
 210 215 220  
 45 Trp Leu Arg Pro Val His Arg Gln Leu Gly Glu Ala Asn Glu Glu Phe  
 225 230 235 240  
 Ala Leu Arg Val Gln Gln Leu Val Ala Lys Glu Leu Gly Gln Thr Gly  
 245 250 255  
 50 Thr Arg Leu Thr Pro Ala Asp Lys Ala Glu His Met Lys Arg Gln Arg  
 260 265 270  
 55 His Pro Arg Leu Arg Pro Gln Ser Ala Gln Ser Ser Phe Pro Pro Ser  
 275 280 285  
 Pro Gly Pro Ser Pro Asp Val Gln Leu Ala Thr Leu Ala Gln Arg Val  
 290 295 300  
 60

119

Lys Glu Val Leu Pro His Val Pro Leu Gly Val Ile Gln Arg Asp Leu  
 305 310 315 320  
 5 Ala Lys Thr Gly Cys Val Asp Leu Thr Ile Thr Asn Leu Leu Glu Gly  
 325 330 335  
 Ala Val Ala Phe Met Pro Glu Asp Ile Thr Lys Gly Thr Gln Ser Leu  
 340 345 350  
 10 Pro Thr Ala Ser Ala Ser Lys Phe Pro Ser Ser Gly Pro Val Thr Pro  
 355 360 365  
 Gln Pro Thr Ala Leu Thr Phe Ala Lys Ser Ser Trp Ala Arg Gln Glu  
 370 375 380  
 15 Ser Leu Gln Glu Arg Lys Gln Ala Leu Tyr Glu Tyr Ala Arg Arg Arg  
 385 390 395 400  
 20 Phe Thr Glu Arg Arg Ala Gln Glu Ala Asp  
 405 410

25 (2) INFORMATION FOR SEQ ID NO: 36:  
 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 25 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear  
 30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

Met Val Glu Leu Ala Ser Cys Asn Ser Pro Phe Ser Phe Leu Pro Leu  
 1 5 10 15  
 35 Ser Leu Pro Ala Phe Pro Ile Leu Leu  
 20 25

40 (2) INFORMATION FOR SEQ ID NO: 37:  
 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 37 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear  
 45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:

Met Leu Phe Pro Ile Leu Leu Gln Thr Met Phe Ser Ala Tyr Leu Gly  
 1 5 10 15  
 50 Ser Glu Gln Tyr Lys Leu Leu Ile Lys Ala Leu Gln Leu Ser Glu Pro  
 20 25 30  
 Gly Lys Glu Ile His  
 55 35

60 (2) INFORMATION FOR SEQ ID NO: 38:

120

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 64 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

## 5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

Met Lys Val Leu Ile Ser Ser Leu Leu Leu Leu Pro Leu Met Leu  
 1 5 10 15

10 Met Ser Met Val Ser Ser Ser Leu Asn Pro Gly Val Ala Arg Gly His  
 20 25 30

Arg Asp Arg Gly Gln Xaa Ser Arg Arg Trp Leu Gln Glu Gly Gly Gln  
 35 40 45

15 Glu Cys Xaa Cys Lys Asp Trp Phe Leu Arg Ala Arg Glu Glu Asn Ser  
 50 55 60

20

## (2) INFORMATION FOR SEQ ID NO: 39:

25

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 155 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

## 30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 39:

Met Cys Cys Ser Leu Ser Met Ile Gly Ala Ala Ser Ser Pro Ser Ser  
 1 5 10 15

35 Gly Cys Ser Ala Arg Ala Arg Ala Ser Ser Gly Leu Pro Trp Leu Trp  
 20 25 30

Gln Pro Cys Pro Gly Pro Arg Phe Arg Cys Ser Leu Trp Met Arg Arg  
 35 40 45

40 Ser Gln Ile Val Ala Pro Ser Thr Cys Ala Pro Arg Ser Gly Ala Xaa  
 50 55 60

45 Gly Leu Ala Val Gly Cys Gly Ala Ile Gly Ala Leu Val Leu Gly Ala  
 65 70 75 80

Gly Leu Leu Phe Ser Leu Arg Ser Val Arg Ser Val Val Leu Arg Ala  
 85 90 95

50 Gly Gly Gln Gln Val Thr Leu Thr Thr His Ala Pro Phe Gly Leu Gly  
 100 105 110

Ala His Phe Thr Val Pro Leu Lys Gln Val Ser Cys Met Ala His Arg  
 115 120 125

55 Gly Glu Val Pro Ala Met Leu Pro Leu Lys Xaa Lys Gly Arg Arg Phe  
 130 135 140

60 Tyr Phe Leu Leu Asp Lys Thr Gly His Phe Pro  
 145 150 155

## (2) INFORMATION FOR SEQ ID NO: 40:

5

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 119 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

10

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 40:

Met Thr Val Tyr Ala Leu Val Val Val Ser Tyr Phe Leu Ile Thr Gly  
 1 5 10 15

15 Gly Ile Ile Tyr Asp Val Ile Val Glu Pro Pro Ser Val Gly Ser Met  
 20 25 30

Thr Asp Glu His Gly His Gln Arg Pro Val Ala Phe Leu Ala Tyr Arg  
 35 40 45

20

Val Asn Gly Gln Tyr Ile Met Glu Gly Leu Ala Ser Ser Phe Leu Phe  
 50 55 60

25 Thr Met Gly Gly Leu Gly Phe Ile Ile Leu Asp Arg Ser Asn Ala Pro  
 65 70 75 80

Asn Ile Pro Lys Leu Asn Arg Phe Leu Leu Leu Phe Ile Gly Phe Val  
 85 90 95

30 Cys Val Leu Leu Ser Phe Phe Met Ala Arg Val Phe Met Arg Met Lys  
 100 105 110

Leu Pro Gly Tyr Leu Met Gly  
 115

35

## (2) INFORMATION FOR SEQ ID NO: 41:

40

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 56 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 41:

45

Met Glu Ser Gly His Leu Leu Trp Ala Leu Leu Phe Met Gln Ser Leu  
 1 5 10 15

50 Trp Pro Gln Leu Thr Asp Gly Ala Thr Arg Val Tyr Tyr Leu Gly Ile  
 20 25 30

Arg Asp Val Gln Trp Asn Tyr Ala Pro Lys Gly Arg Asn Val Ile Thr  
 35 40 45

55 Asn Gln Pro Leu Asp Ser Asp Met  
 50 55

60

## (2) INFORMATION FOR SEQ ID NO: 42:

122

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 109 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 42:

5 Met Lys Asp Phe Asn His Ser Tyr His Ala Cys Gly Val Ile Ala Thr  
 1 5 10 15  
 10 Ile Ala Phe Leu Met Ile Asn Ala Val Ser Asn Gly Gln Val Arg Gly  
 20 25 30  
 15 Asp Ser Tyr Ser Glu Gly Cys Leu Gly Gln Thr Gly Ala Arg Ile Trp  
 35 40 45  
 Leu Phe Val Gly Phe Met Leu Ala Phe Gly Ser Leu Ile Ala Ser Met  
 50 55 60  
 20 Trp Ile Leu Phe Gly Gly Tyr Val Ala Lys Glu Lys Asp Ile Val Tyr  
 65 70 75 80  
 Pro Gly Ile Ala Val Phe Phe Gln Asn Ala Phe Ile Phe Phe Gly Gly  
 85 90 95  
 25 Leu Val Phe Lys Phe Gly Arg Thr Glu Asp Leu Trp Gln  
 100 105

30

## (2) INFORMATION FOR SEQ ID NO: 43:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 69 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 43:

35 Met Val Thr Ile Phe Asn Ile Ile Thr Thr Thr Thr Ile Leu Pro Thr  
 40 1 5 10 15  
 Gln Thr Ala Ala Ala Pro Pro Ser Trp Thr Pro Ala Val Cys Ser Leu  
 20 25 30  
 45 Ser Ser Trp Pro Gly Ser Pro Arg Ser Trp Ala Gly Pro Val Leu Arg  
 35 40 45  
 Asp Ser Ala Arg Arg Cys Ala Trp Asn Ser Trp Thr Thr Arg Ala Asp  
 50 55 60  
 50 Pro Ser Ser Ala Met  
 65

55

## (2) INFORMATION FOR SEQ ID NO: 44:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 67 amino acids

(B) TYPE: amino acid

60



123

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 44:

5 Met Gly Lys Cys Leu Cys Arg Gly Ala Glu Leu Ser Leu Cys Phe Ser  
 1 5 10 15  
 Phe Phe Pro Leu Leu Leu Pro Leu His Thr Pro Val Ala Gly Arg Asn  
 20 25 30  
 10 Leu Gly Phe Pro Glu Ser Leu Gly Val Pro Pro Phe Leu Pro His Pro  
 35 40 45  
 Gly Gly Thr Pro Arg Ala Pro Gly Leu Phe Leu Leu Leu Phe Ser Phe  
 50 55 60  
 15 Trp Ala Val  
 65

20

(2) INFORMATION FOR SEQ ID NO: 45:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 41 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 45:

30 Met Leu Leu Leu Met Ile Leu Glu Xaa Phe Pro Cys Pro Gln Pro Gly  
 1 5 10 15  
 Thr Asn Val Ile Ile Ile Ser Met Cys Phe Phe Leu Ser His Ser Asn  
 20 25 30  
 35 Ala Ile Tyr Leu Gln His Tyr His Phe  
 35 40

40

(2) INFORMATION FOR SEQ ID NO: 46:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 9 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

45

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 46:

50 Met Leu Tyr Leu Leu Tyr Leu Ala Phe  
 1 5

(2) INFORMATION FOR SEQ ID NO: 47:

55

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 47 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

60

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 47:

124

Met Gly Asn Cys His Asp His Arg Gly Leu Glu Phe Cys Phe Phe Phe  
 1 5 10 15  
 Phe Phe Phe Phe Phe Gly Gly Leu Gly Asp Ser Ser Gln Asp His Ile  
 20 25 30  
 Leu Ala Ser Phe Ser Pro Thr Pro Phe Arg Lys Ile Ser Asn Arg  
 35 40 45

10

(2) INFORMATION FOR SEQ ID NO: 48:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 49 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 48:

Met His His His Thr Leu Leu Ile Phe Val Phe Leu Val Glu Thr Ala  
 1 5 10 15  
 Phe His His Leu Gly Gln Thr Gly Leu Lys Leu Leu Ala Ser Ser Asp  
 20 25 30  
 Ser Ser Ala Ser Ala Ser Gln Lys Lys Lys Lys Lys Lys Lys Asn  
 35 40 45  
 Ser

30

(2) INFORMATION FOR SEQ ID NO: 49:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 46 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 49:

Met His Gln His Ala Gln Leu Ser Ile Leu Phe Leu Val Glu Ile Arg  
 1 5 10 15  
 Ser Cys Cys Val Ala Gln Ala Ser Leu Lys Phe Leu Ala Ser Ser Asn  
 20 25 30  
 Pro Ser Ala Leu Ala Ser Gln Ile Val Gly Phe Xaa Arg His  
 35 40 45

50

(2) INFORMATION FOR SEQ ID NO: 50:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 30 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 50:

60

125

Met Asp His Ser Pro Phe Ala Gly Ile Asn Asn Thr Ala Val Leu Val  
 1 5 10 15

5 Leu Thr Leu His Phe His His Ala Thr Leu Ser Val Thr Glu  
 20 25 30

(2) INFORMATION FOR SEQ ID NO: 51:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 35 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 51:

Cys Ala Gly Cys Asp Glu Leu Ile Phe Ser Asn Glu Tyr Thr Gln Ala  
 1 5 10 15

20 Glu Asn Gln Asn Trp His Leu Lys His Phe Cys Cys Phe Asp Cys Asp  
 20 25 30

Ser Ile Leu  
 35

25

(2) INFORMATION FOR SEQ ID NO: 52:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 159 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 52:

35 Ala Arg Gly Phe Val Cys Ser Thr Cys His Glu Leu Leu Val Asp Met  
 1 5 10 15

40 Ile Tyr Phe Trp Lys Asn Glu Lys Leu Tyr Cys Gly Arg His Tyr Cys  
 20 25 30

Asp Ser Glu Lys Pro Arg Cys Ala Gly Cys Asp Glu Leu Ile Phe Ser  
 35 40 45

45 Asn Glu Tyr Thr Gln Ala Glu Asn Gln Asn Trp His Leu Lys His Phe  
 50 55 60

Cys Cys Phe Asp Cys Asp Ser Ile Leu Ala Gly Glu Ile Tyr Val Met  
 65 70 75 80

50 Val Asn Asp Lys Pro Val Cys Lys Pro Cys Tyr Val Lys Asn His Ala  
 85 90 95

Val Val Cys Gln Gly Cys His Asn Ala Ile Asp Pro Glu Val Gln Arg  
 100 105 110

Val Thr Tyr Asn Asn Phe Ser Trp His Ala Ser Thr Glu Cys Phe Leu  
 115 120 125

60 Cys Ser Cys Cys Ser Lys Cys Leu Ile Gly Gln Lys Phe Met Pro Val

126

130

135

140

Glu Gly Met Val Phe Cys Ser Val Glu Cys Lys Lys Arg Met Ser  
 145 150 155

5

(2) INFORMATION FOR SEQ ID NO: 53:

10

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 93 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 53:

15

Gly Val Ala Arg Gly His Arg Asp Arg Gly Gln Ala Ser Arg Arg Trp  
 1 5 10 15

20

Leu Gln Glu Gly Gly Gln Glu Cys Glu Cys Lys Asp Trp Phe Leu Arg  
 20 25 30

Ala Pro Arg Arg Lys Phe Met Thr Val Ser Gly Leu Pro Lys Lys Gln  
 35 40 45

25

Cys Pro Cys Asp His Phe Lys Gly Asn Val Lys Lys Thr Arg His Gln  
 50 55 60

30

Arg His His Arg Lys Pro Asn Lys His Ser Arg Ala Cys Gln Gln Phe  
 65 70 75 80

Leu Lys Gln Cys Gln Leu Arg Ser Phe Ala Leu Pro Leu  
 85 90

35

(2) INFORMATION FOR SEQ ID NO: 54:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 12 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 54:

40

His Thr Gln Val Glu Phe Ile Pro Arg Met Gln Cys  
 1 5 10

45

(2) INFORMATION FOR SEQ ID NO: 55:

50

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 16 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 55:

55

Leu Lys Ile Arg Lys Pro Ile Asn Val Ile Tyr His Ile Asn Arg Leu  
 1 5 10 15

60

127

## (2) INFORMATION FOR SEQ ID NO: 56:

5

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 16 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 56:

10

Arg Lys Met Gly Ile Glu Arg Asn Phe His Gln Ser Gly Lys Gly Ile  
 1 5 10 15

15

## (2) INFORMATION FOR SEQ ID NO: 57:

20

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 57:

25

Lys Val Pro Thr Ala Asn Ile Ile Leu Asn Gly Glu Arg Leu Asn Ala  
 1 5 10 15

Phe Pro Ile Arg Thr  
 20

30

## (2) INFORMATION FOR SEQ ID NO: 58:

35

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 28 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 58:

40

Ile Phe Ser Ser Val Leu His Ser Phe Gln Tyr Thr Asn Pro Val Pro  
 1 5 10 15

Phe Phe Phe Arg Phe Thr Pro Ser Thr Leu Phe Phe  
 20 25

45

## (2) INFORMATION FOR SEQ ID NO: 59:

50

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

55

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 59:

Lys Val Pro Thr Ala Asn Ile Ile Leu Asn Gly Glu Arg Leu Asn Ala  
 1 5 10 15

60

Phe Pro Ile Arg Thr

128

20

## 5 (2) INFORMATION FOR SEQ ID NO: 60:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

10

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 60:

Met Tyr Phe Leu Ser Ser Leu Leu Ile His Glu His Val Ile Ser Val  
 1 5 10 15

15

Ile Phe Ser Ile Leu  
 20

20

## (2) INFORMATION FOR SEQ ID NO: 61:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 28 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

25

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 61:

Glu Asp Gly Ser Ala Pro Arg Glu Gly Glu Thr Ser Ala Pro Arg Leu  
 1 5 10 15

30

Pro Glu Val Val Arg Ile Thr Ser Ala Gly Ile Cys  
 20 25

129

Applicant's or agent's file reference number	PZ005PCT	International application No.	Unassigned
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## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>19</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT	
Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) American Type Culture Collections 10801 University Blvd. Manassas, VA 20110-2209 United States of America	
Date of deposit	March 13, 1997
Accession Number	97955
C. ADDITIONAL INDICATIONS (leave blank if not applicable)	
This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (If the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")	

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Authorized officer <i>P. Williams</i>	

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130

Applicant's or agent's file reference number	2005PCT	International application No. <del>Unassigned</del>
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B. IDENTIFICATION OF DEPOSIT	
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Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) American Type Culture Collections 10801 University Blvd. Manassas, VA 20110-2209 United States of America	
Date of deposit May 22, 1997	Accession Number 209074
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (If the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
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***What Is Claimed Is:***

1. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 95% identical to a sequence selected from the group consisting of:
- 5 (a) a polynucleotide fragment of SEQ ID NO:X or a polynucleotide fragment of the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X;
- (b) a polynucleotide encoding a polypeptide fragment of SEQ ID NO:Y or a polypeptide fragment encoded by the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X;
- 10 (c) a polynucleotide encoding a polypeptide domain of SEQ ID NO:Y or a polypeptide domain encoded by the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X;
- (d) a polynucleotide encoding a polypeptide epitope of SEQ ID NO:Y or a polypeptide epitope encoded by the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X;
- 15 (e) a polynucleotide encoding a polypeptide of SEQ ID NO:Y or the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X, having biological activity;
- 20 (f) a polynucleotide which is a variant of SEQ ID NO:X;
- (g) a polynucleotide which is an allelic variant of SEQ ID NO:X;
- (h) a polynucleotide which encodes a species homologue of the SEQ ID NO:Y;
- (i) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(h), wherein said polynucleotide does not hybridize under stringent conditions to a nucleic acid molecule having a nucleotide sequence of only A residues or of only T residues.
- 25
2. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises a nucleotide sequence encoding a secreted protein.
- 30
3. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises a nucleotide sequence encoding the sequence identified as SEQ ID NO:Y or the polypeptide encoded by the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X.
- 35

4. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises the entire nucleotide sequence of SEQ ID NO:X or the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X.
5. The isolated nucleic acid molecule of claim 2, wherein the nucleotide sequence comprises sequential nucleotide deletions from either the C-terminus or the N-terminus.
6. The isolated nucleic acid molecule of claim 3, wherein the nucleotide sequence comprises sequential nucleotide deletions from either the C-terminus or the N-terminus.
7. A recombinant vector comprising the isolated nucleic acid molecule of claim 1.
8. A method of making a recombinant host cell comprising the isolated nucleic acid molecule of claim 1.
9. A recombinant host cell produced by the method of claim 8.
10. The recombinant host cell of claim 9 comprising vector sequences.
11. An isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence selected from the group consisting of:
- (a) a polypeptide fragment of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z;
  - (b) a polypeptide fragment of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z, having biological activity;
  - (c) a polypeptide domain of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z;
  - (d) a polypeptide epitope of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z;
  - (e) a secreted form of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z;
  - (f) a full length protein of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z;

- (g) a variant of SEQ ID NO:Y;
- (h) an allelic variant of SEQ ID NO:Y; or
- (i) a species homologue of the SEQ ID NO:Y.

5       12.     The isolated polypeptide of claim 11, wherein the secreted form or the full length protein comprises sequential amino acid deletions from either the C-terminus or the N-terminus.

10       13.     An isolated antibody that binds specifically to the isolated polypeptide of claim 11.

14       14.     A recombinant host cell that expresses the isolated polypeptide of claim 11.

15       15.     A method of making an isolated polypeptide comprising:  
      (a) culturing the recombinant host cell of claim 14 under conditions such that said polypeptide is expressed; and  
      (b) recovering said polypeptide.

20       16.     The polypeptide produced by claim 15.

17.     A method for preventing, treating, or ameliorating a medical condition, comprising administering to a mammalian subject a therapeutically effective amount of the polypeptide of claim 11 or the polynucleotide of claim 1.

25       18.     A method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject comprising:  
      (a) determining the presence or absence of a mutation in the polynucleotide of claim 1; and  
      (b) diagnosing a pathological condition or a susceptibility to a pathological  
30     condition based on the presence or absence of said mutation.

19.     A method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject comprising:  
      (a) determining the presence or amount of expression of the polypeptide of  
35     claim 11 in a biological sample; and  
      (b) diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or amount of expression of the polypeptide.

20. A method for identifying a binding partner to the polypeptide of claim 11 comprising:
- 5 (a) contacting the polypeptide of claim 11 with a binding partner; and  
(b) determining whether the binding partner effects an activity of the polypeptide.
21. The gene corresponding to the cDNA sequence of SEQ ID NO:Y.
- 10 22. A method of identifying an activity in a biological assay, wherein the method comprises:
- (a) expressing SEQ ID NO:X in a cell;  
(b) isolating the supernatant;  
(c) detecting an activity in a biological assay; and  
15 (d) identifying the protein in the supernatant having the activity.
23. The product produced by the method of claim 22.

Applicant's or agent's file reference number	2005PCT	International application	Unassigned
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PCT/US 98/06801

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Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) American Type Culture Collections 10801 University Blvd. Manassas, VA 20110-2209 United States of America	
Date of deposit	March 13, 1997
Accession Number	97955
C. ADDITIONAL INDICATIONS (leave blank if not applicable)	
This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4)EPC).	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")	

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Authorized officer	Authorized officer

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**CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

**NORWAY**

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

**AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

**FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

**UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

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**DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

**SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

**NETHERLANDS**

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

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